

Distinct physiological adaptation strategies of bread wheat genotypes under simultaneous salinity and *Gaeumannomyces tritici* stress: the role of ionic homeostasis and antioxidant regulation

Mozhgan Gholizadeh Vazvani

Vali-e-Asr University of Rafsanjan

Roohallah Saberi Riseh

r.saberi@vru.ac.ir

Vali-e-Asr University of Rafsanjan

Hossein Dashti

Vali-e-Asr University of Rafsanjan

Evelin Loit-Harro

Estonian University of Life Sciences

Research Article

Keywords: Wheat, combined abiotic–biotic stress, salinity tolerance, take-all disease, ionic homeostasis, antioxidant defense, phenylpropanoid pathway, physiological adaptation strategy

Posted Date: August 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10560836/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Distinct physiological adaptation strategies of bread wheat genotypes under simultaneous salinity and *Gaeumannomyces tritici* stress: the role of ionic homeostasis and antioxidant regulation

Mozhgan Gholizadeh Vazvani¹, Roohallah Saberi Riseh^{1*}, Hossein Dashti², Evelin Loit-Harro³

¹: Department of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, 7718897111 Rafsanjan, Iran.

²: Department of Genetic and Plant Production, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, 7718897111 Rafsanjan, Iran.

³: Chair of Crop Science, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006, Tartu, Estonia.

*: corresponding author, Email: r.saberi@vru.ac.ir

Abstract

Background: Combined abiotic and biotic stresses frequently occur in agricultural systems, yet the physiological mechanisms determining crop performance under stress combinations remain insufficiently understood. Salinity and take-all disease caused by *Gaeumannomyces tritici* are major constraints for wheat production, particularly in salt-affected regions. This study aimed to identify physiological indicators and adaptive strategies associated with wheat performance under simultaneous salinity and pathogen stress.

Results: Ten bread wheat (*Triticum aestivum* L.) genotypes were evaluated under control, salinity, *G. tritici* infection, and combined salinity–pathogen conditions using a factorial greenhouse experiment with three replications. Root and shoot K^+/Na^+ ratios, peroxidase (POD) activity, phenylalanine ammonia-lyase (PAL) activity, and total soluble protein content were analyzed. Analysis of variance revealed highly significant effects of genotype, treatment, and genotype $\times$ treatment interaction for all measured traits ($p < 0.001$), indicating strong genetic variation in stress adaptation. Under combined stress, genotypes 1528 and 1642 maintained superior ionic balance and antioxidant capacity, particularly through higher K^+/Na^+ ratios and POD activity. Principal component analysis showed that the first two components explained 81.8% of total trait variation under combined stress, separating genotypes according to ionic–antioxidant regulation versus metabolic defense strategies. Ward’s cluster analysis further separated 30 genotype–environment combinations into two distinct groups. The first cluster (19 observations) was characterized by positive standardized values for root K^+/Na^+ ratio (+0.657), shoot K^+/Na^+ ratio (+0.894), and POD activity (+0.395), whereas the second cluster (11 observations) showed higher total protein and PAL activity (+0.558 for both traits), representing contrasting physiological strategies.

Conclusions: The results demonstrate that wheat plants do not rely on a single defense mechanism under combined stress but dynamically shift between ionic–antioxidant regulation and secondary metabolic defense depending on the dominant stress component. Shoot and root K^+/Na^+ ratios, together with POD activity, represent promising physiological indicators for identifying wheat genotypes adapted to saline and disease-prone environments.

Keywords: Wheat; combined abiotic–biotic stress; salinity tolerance; take-all disease; ionic homeostasis; antioxidant defense; phenylpropanoid pathway; physiological adaptation strategy

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and represents a major source of human nutrition and livestock feed. With the global population projected to reach approximately 9.7 billion by 2050, maintaining and improving wheat productivity has become a major challenge for global food security [1, 2].

However, wheat productivity is increasingly threatened by environmental stresses associated with climate change, including water scarcity and soil salinity, which adversely affect plant growth and yield [3].

Factors such as farming practices, weather variability, infrastructure, and technology influence soil quality, with climate change playing a significant role in altering weather patterns and soil conditions [4]. Wheat (*Triticum aestivum* L.) is one of the world's most important crops and is crucial for global food security. Nevertheless, wheat production

remains highly vulnerable to both abiotic and biotic stresses, which frequently occur simultaneously under field conditions. Among abiotic stresses, soil salinity is one of the major constraints limiting wheat production, particularly in arid and semi-arid regions, where it disrupts plant growth through osmotic stress, ion toxicity, and nutrient imbalance [5].

Salinity reduces plant growth and yield through osmotic stress, ionic toxicity, and impaired uptake of essential nutrients such as K^+ [5]. Among biotic stresses, take-all disease caused by the soil-borne fungus *Gaeumannomyces tritici* is considered the most important root disease of wheat worldwide. The pathogen colonizes wheat roots, spreads through root tissues, and damages vascular structures, thereby impairing water and nutrient uptake and causing substantial yield losses [6-8].

Plants have evolved several mechanisms to cope with salt stress, including ionic regulation, osmotic adjustment, and antioxidant defense systems [5]. The K^+/Na^+ ratio in plant tissues is widely recognized as a key indicator of salt tolerance [9]. Tolerant genotypes limit Na^+ uptake while maintaining K^+ absorption, thereby preserving a favorable K^+/Na^+ balance. This regulation occurs at both the root level (restricting Na^+ entry) and the shoot level [10]. Previous studies have shown that salt-tolerant wheat genotypes maintain higher shoot K^+/Na^+ ratios under saline conditions ([11].

In addition to ionic regulation, plants activate their antioxidant defense systems in response to both biotic and abiotic stresses. Environmental stresses trigger the overproduction of reactive oxygen species (ROS), which can damage cell membranes and macromolecules [12]. Antioxidant enzymes such as peroxidase (POD) play a vital role in scavenging ROS and mitigating oxidative damage [13]. Increased POD activity under salt stress has been reported in tolerant genotypes [14], and enhanced peroxidase activity is also a well-documented early defense response to pathogen infection [15].

Plants also activate the phenylpropanoid metabolic pathway in response to biotic stress. The enzyme phenylalanine ammonia-lyase (PAL) is the key entry-point enzyme in this pathway, catalyzing the synthesis of phenolic compounds, phytoalexins, and lignin [16]. Increased PAL activity following fungal infection has been documented in wheat [17] and some evidence suggests that PAL may also play a role under salt stress [18].

PAL is recognized as a key metabolic valve that redirects carbon flux between primary metabolism and the phenylpropanoid pathway [19]. While PAL activity is strongly induced upon pathogen attack [17], it is generally suppressed under salinity stress, as plants prioritize energy-efficient ionic regulation over costly secondary metabolic defense pathways [20, 21].

In field conditions, plants are often exposed to combined abiotic and biotic stresses simultaneously [22]. However, most studies have focused on individual stresses, and relatively little research has addressed the combined effects of salinity and disease in wheat. Responses to combined stresses are complex and often unpredictable because distinct signaling pathways may interact synergistically or antagonistically [23, 24]. The outcome of such interactions depends on multiple factors, including the type, intensity, and duration of each stress, as well as the plant species and developmental stage [24].

Evidence indicates that the impact of salinity on fungal disease development can be dual and sometimes contradictory. For instance, a study conducted more than two decades ago found that the severity of maize susceptibility to smut disease (*Ustilago maydis*) diminished when salinity increased to 9 dS m^{-1} [25]. In another study on tomato, low salt concentrations (50 mM NaCl) suppressed the plant's defense performance against powdery mildew (*Oidium neolycopersici*), whereas higher salt concentrations (150 mM NaCl) enhanced resistance to the same pathogen [26]. Conversely, low to moderate NaCl levels (50 or 100 mM) can increase plant susceptibility to fungal stress, as observed in tomato crops under mild salinity stress [26]. Positive interactions have also been documented; for example, salt-induced osmotic stress improved resistance against powdery mildew in barley through enhanced antioxidant activity [27]. Furthermore, exposure to one stress can sometimes confer tolerance to another unrelated stress, a phenomenon known as cross-tolerance [28, 29].

From a molecular perspective, salinity stress, through increased abscisic acid (ABA) accumulation, can antagonize salicylic acid (SA) signaling and suppress the expression of defense-related genes, thereby compromising resistance to biotrophic pathogens under combined stress conditions [30, 31]. Conversely, salt-induced osmotic adjustment may enhance resistance to some foliar pathogens by promoting stomatal closure or accumulating antifungal ions [26, 32]. These observations underscore the complexity of plant responses to combined stresses and highlight the need for genotype-specific studies under realistic field conditions.

The responses of plants to biotic and abiotic stress combinations are inherently complex, as the multitude of interactions involves two living organisms—the plant and the pathogen—under the additional dimension of abiotic stress exposure [24]. Evidence indicates that physiological and molecular responses of plants to combined abiotic–pathogen stresses are significantly different from their responses to individual stresses [31, 33]. Understanding the

responses of diverse wheat genotypes to combined salinity and take-all disease is therefore of particular importance for breeding programs.

Given that the world is facing severe climate change impacts, and that take-all disease acts as a major growth-limiting constraint for wheat, this study is the first to investigate the combined effects of salinity and this pathogen on wheat physiological and ionic responses. Identifying reliable physiological markers that can predict performance under concurrent stresses would greatly assist breeders in selecting superior genotypes. Given this research gap, the main objectives of this study were: (1) to identify physiological indices and their combinations that best differentiate wheat genotypes for combined tolerance to salinity and take-all disease; (2) to evaluate Na⁺ and K⁺ concentrations in roots and shoots, activities of antioxidant enzymes (POD and PAL), and total protein content in ten wheat genotypes under four environmental conditions (control, salinity, pathogen inoculation, and combined salinity–pathogen stress); and (3) to use principal component analysis (PCA) and cluster analysis to determine the contribution of each trait to genotype discrimination under each stress condition.

2. Materials and Methods

2.1. Plant Materials and treatments

Ten bread wheat (*Triticum aestivum* L.) genotypes were selected based on a previous screening of 100 genotypes evaluated for resistance to take-all disease (*Gaeumannomyces tritici*). In that earlier study, cluster analysis using disease severity, lignin content, and root weight categorized the genotypes into susceptible, moderately resistant, and resistant groups [34, 35]. To represent the full spectrum of responses, three susceptible genotypes (164, Kavir, and Sardari), four moderately resistant genotypes (862, Roshan, 9033, and 9056), and three resistant genotypes (1528, 1642, and 2071) were selected for the present investigation.

The experiment was conducted under greenhouse conditions using a factorial arrangement in a completely randomized design (CRD) with three replications. The greenhouse was maintained at day/night temperatures of 25 ± 2 °C and 18 ± 2 °C, respectively, with a relative humidity of 60–70% and a photoperiod of 16 h light/8 h dark. Seeds were surface-sterilized with 1% sodium hypochlorite for 5 min, rinsed thoroughly with sterile distilled water, and sown (three seeds per pot) in plastic pots containing 1 kg of washed sterilized sand. The sand was previously characterized for pH (7.84) and electrical conductivity (EC; 1.75 mS cm⁻¹). Plants were irrigated with half-strength Hoagland nutrient solution once a week throughout the experiment to provide essential mineral nutrients. The same nutrient regime was applied to all treatments, including control, salinity, pathogen inoculation, and combined salinity–pathogen stress. The soil was autoclaved at 121 °C for 1 h on three consecutive days to ensure uniformity and eliminate any background microflora. Plants were irrigated with half-strength Hoagland nutrient solution throughout the experiment to ensure adequate mineral nutrition. The same nutrient regime was applied to all treatments, including control, salinity, pathogen inoculation, and combined stress treatments. The experiment consisted of four treatment groups: (1) control (non-stressed), (2) salinity stress, (3) pathogen inoculation, and (4) combined salinity–pathogen stress. Plants were grown under these conditions until the two-leaf stage, when stress treatments were initiated. All treatments were maintained for four weeks until final harvest.

2.2. Salinity Stress Treatment

At the two-leaf stage, salinity stress was imposed by irrigating plants once with NaCl solution to establish a final concentration of 150 mM NaCl in the sand culture. This concentration has been effectively used for screening wheat genotypes in sand culture, enabling clear differentiation between salt-tolerant and salt-sensitive cultivars [36]. Following salt application, plants were irrigated with non-saline water, and half-strength Hoagland nutrient solution was supplied once a week throughout the remaining experimental period. Salt accumulation was monitored every three days by measuring the electrical conductivity (EC) of the sand extract.

2.3. Pathogen Inoculation

At the two-leaf stage, plants assigned to the pathogen and combined stress treatments were inoculated with the *Gaeumannomyces tritici* isolate used in the previous screening study [34, 35]. The fungal inoculum was prepared using a colonized millet–sand substrate containing actively growing mycelium of *G. tritici*. Briefly, millet seeds and sand (1:1, w/w) were thoroughly mixed, moistened with distilled water, and sterilized by autoclaving at 121 °C for 1 h on two successive days. The sterilized substrate was then inoculated with fresh mycelial plugs of *G. tritici* and

incubated at 25 °C for two weeks to allow complete fungal colonization. The identity and pathogenicity of the fungal isolate were confirmed according to the protocol described in the previous screening study [34, 35]. For inoculation, the sand around the crown region of each plant was carefully removed, and the millet–sand–fungus inoculum was applied at a rate of 0.5% (w/w) directly to the root zone. The sand was then gently replaced to ensure uniform contact between the roots and the inoculum. In the control and salinity-only treatments, the same amount of sterilized millet–sand mixture without the fungus was applied to maintain identical substrate disturbance conditions. Plants were maintained under greenhouse conditions for four weeks after inoculation before physiological and biochemical measurements were performed.

2.4. Combined Stress Treatment

For the combined stress treatment, salinity and pathogen inoculation were applied at the same developmental stage (two-leaf stage). Plants were first irrigated with 150 mM NaCl solution as described in Section 2.2, followed immediately by inoculation with *G. tritici* as described in Section 2.3. This approach was designed to establish overlapping abiotic and biotic stress conditions and to evaluate wheat responses under combined stress exposure. All plants were maintained under the corresponding treatment conditions for four weeks until harvest.

2.5. Confirmation of Infection

Infection symptoms were confirmed at the end of the experiment by visual examination of root and crown tissues. Characteristic blackening (necrosis) and discoloration of vascular tissues were used as diagnostic symptoms of take-all disease. Successful infection was confirmed at the end of the experiment by visual examination of roots and crown tissues. The characteristic blackening and discoloration of vascular tissues were used as diagnostic symptoms of take-all disease. The observed symptoms were consistent with the previously established response classification of the selected genotypes under the same inoculation conditions. [34, 35]. Disease severity was not considered a response variable in the present study because the main objective was to characterize host physiological and ionic responses under a standardized pathogen challenge

2.6. Physiological and Biochemical Measurements

2.6.1. Determination of Potassium (K⁺) and Sodium (Na⁺) Concentrations in Roots and Shoots

After four weeks of stress exposure, shoots and roots were collected separately and briefly washed with distilled water to remove surface-adhered ions. Samples were dried in a forced-air oven at 72 °C for 48 h until constant weight. Dried samples (0.5 g) were ground to a fine powder using a mortar and pestle and transferred to porcelain crucibles. Samples were subjected to dry ashing at 250 °C for 2 h followed by 550 °C for 3 h in a muffle furnace. After cooling, the ash was dissolved in 5 mL of 2 N hydrochloric acid (HCl). The solution was filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask and adjusted to volume with double-distilled water. Potassium and sodium concentrations were determined using a flame photometer, and the K⁺/Na⁺ ratio was calculated for root and shoot tissues.

2.6.2. Biochemical Assays (Protein, PAL, and POD)

Fresh leaf tissue (0.5 g) was homogenized in 3–5 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.5) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 4,000 rpm for 20–30 min at 4 °C, and the resulting supernatant (crude enzyme extract) was stored at –20 °C for further analyses. Total soluble protein concentration in the extract was determined using the Bradford method [37], with bovine serum albumin (BSA) as the standard.

PAL activity was measured spectrophotometrically by quantifying the rate of conversion of L-phenylalanine to trans-cinnamic acid, according to the method described by D'Cunha et al. (1996) with minor modifications [38]. The reaction mixture consisted of 1.0 mL of the same potassium phosphate extraction buffer (50 mM, pH 7.5), 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of double-distilled water, and 0.1 mL of the crude enzyme extract. The mixture was incubated at 37 °C for 1 h, and the reaction was terminated by adding 0.5 mL of 6 M HCl. The absorbance of the final solution was read at 260 nm using a UV-Vis spectrophotometer. Enzyme activity was calculated using a standard curve

prepared with authentic trans-cinnamic acid at concentrations of 0, 10, 15, 20, 25, 30, and 35 μM . One unit of PAL activity was defined as 1 μmol of cinnamic acid produced per minute per milligram of protein.

Guaiacol peroxidase activity was assayed according to the procedure of Plewa et al. (1991). The reaction mixture contained 2.77 mL of 50 mM potassium phosphate buffer (pH 7.0), 100 μL of 1% (v/v) H_2O_2 , 100 μL of 4% (w/v) guaiacol, and 30 μL of the crude enzyme extract [39]. The increase in absorbance due to the oxidation of guaiacol to tetraguaiacol was monitored at 470 nm for 3 min using a UV-Vis spectrophotometer. The enzyme activity was calculated using the extinction coefficient of tetraguaiacol ($5.25 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as μmol tetraguaiacol formed per minute per milligram of protein.

2.7. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure for a factorial experiment arranged in a completely randomized design (CRD) with three biological replications. Genotype and stress treatment were considered as fixed factors. The sources of variation included genotype, stress treatment, and genotype $\times$ treatment interaction. Principal component analysis (PCA) was conducted separately for each stress environment to identify the physiological traits contributing most to genotype discrimination and to visualize genotype distribution under each condition. Principal component analysis (PCA) was conducted separately for each treatment environment to identify the most discriminating physiological traits and to visualize genotype distributions. Additionally, cluster analysis based on Ward's linkage method and squared Euclidean distance was performed to classify genotypes according to their overall similarity in physiological and biochemical traits under different stress environments. All statistical analyses and graphical outputs were performed using MINITAB software version 18 (MINITAB Inc., State College, PA, USA).

3. Results

3.1. Analysis of variance

Analysis of variance revealed significant differences among the studied genotypes for all measured traits, including total protein content, POD and PAL activities, and the K^+/Na^+ ratio in shoots and roots (Table 1). The significant genotypic variation indicates substantial differences among wheat genotypes in their physiological and biochemical responses under the tested stress conditions.

Treatment effects were significant for all evaluated traits, indicating that salinity, fungal inoculation, and their combination significantly altered physiological and biochemical parameters. Furthermore, the significant genotype $\times$ treatment interaction demonstrated that genotypes responded differently across the tested environmental conditions.

Table 1- ANOVA results for physiological and biochemical traits evaluated under different environmental conditions

SOV	df	MS				
		K/Na in Root	K/Na in Shoot	PAL	POD	Total protein
Genotype	9	0.0037***	0.0772***	0.000034***	1.5777***	1046.35***
treatment	3	0.0598***	2.0268***	0.000013***	1.0630***	403.97***
Genotype $\times$ Treatment	27	0.0046***	0.0618***	0.000043***	0.8401***	1314.16***
Error experimental	80	0.00032	0.0069	0.000001	0.0457	21.31
Total	119					
CV (%)		16.74	15.42	3.33	20.22	3.33

***: Significant at the 0.001 probability level

3.2. Ionic responses: root and shoot K^+/Na^+ ratios

Significant variation was observed among wheat genotypes for root K^+/Na^+ ratio across different stress conditions (Table 2). Under control conditions, genotype 1642 exhibited the highest root K^+/Na^+ ratio, whereas genotypes 1528, 164, and 2071 showed comparatively lower values. Following *G. tritici* inoculation, root K^+/Na^+ ratios decreased in most genotypes, with values remaining significantly lower than those recorded under control conditions. Under salinity stress, genotypes 164 and 1528 maintained relatively high root K^+/Na^+ ratios compared with several other

genotypes. Under combined salinity and *G. tritici* stress, variation among genotypes was observed; genotypes 1528 and Kavir showed relatively higher root K^+/Na^+ ratios compared with several other entries (Table 2). Shoot K^+/Na^+ ratio also differed significantly among genotypes and treatments (Table 2). Under control conditions, genotypes Sardari and 164 exhibited the highest shoot K^+/Na^+ ratios, whereas other genotypes showed comparable values. Inoculation with *G. tritici* resulted in a significant reduction in shoot K^+/Na^+ ratio in most genotypes, with pronounced decreases observed in genotypes 1642, 1528, 862, and Sardari. Under salinity stress, genotypes 9056 and 862 maintained relatively higher shoot K^+/Na^+ ratios compared with several other genotypes. Under combined salinity and pathogen stress, shoot K^+/Na^+ ratio declined across all genotypes, although the magnitude of reduction differed among entries. Genotypes 862 and 1528 retained comparatively higher values under combined stress conditions (Table 2).

Table 2. Effects of genotype $\times$ treatment interaction on root and shoot K^+/Na^+ ratios of wheat genotypes under control, salinity, *G. tritici* infection, and combined stress conditions.

Treatments	K/Na Root									
	Genotypes									
	9056	9033	2071	1642	1528	862	164	Sardari	Rosha	Kavir
	n									
Control	0.101 ^a _b	0.164 ^a _a	0.094 ^b _b	0.206 ^a	0.076 ^b	0.172 ^a	0.040 ^b _b	0.140 ^a	0.166 ^{ab}	0.127 ^a
<i>G. tritici</i>	0.014 ^c	0.152 ^{ab} _{ab}	0.026 ^c _c	0.038 ^c	0.026 ^c	0.039 ^c	0.030 ^b _b	0.022 ^c	0.034 ^c	0.021 ^b
Salinity	0.090 ^b	0.118 ^b _b	0.152 ^a _a	0.118 ^b	0.170 ^a	0.120 ^{ab}	0.171 ^a _a	0.140 ^a	0.175 ^a	0.114 ^a
Salinity + <i>G. tritici</i>	0.112 ^a	0.152 ^{ab} _{ab}	0.111 ^b _b	0.088 ^{bc}	0.157 ^a	0.104 ^b	0.152 ^a _a	0.090 ^b	0.090 ^{bc}	0.143 ^a

Treatments	K/Na shoot									
	Genotypes									
	9056	9033	2071	1642	1528	862	164	Sardari	Rosha	Kavir
	n									
Control	0.708 ^a	1.022 ^a _a	1.091 ^a _a	0.764 ^a	0.840 ^a	0.905 ^a	1.185 ^a _a	1.269 ^a	0.935 ^a	0.443 ^a
<i>G. tritici</i>	0.364 ^b	0.432 ^b _b	0.415 ^b _b	0.333 ^c	0.269 ^b	0.407 ^c	0.281 ^c _c	0.207 ^b	0.447 ^b	0.273 ^c
Salinity	0.668 ^a	0.339 ^b _b	0.457 ^b _b	0.564 ^b	0.348 ^b	0.682 ^b	0.571 ^b _b	0.431 ^b	0.590 ^b	0.399 ^b
Salinity + <i>G. tritici</i>	0.427 ^b	0.432 ^b _b	0.399 ^b _b	0.446 ^c	0.454 ^b	0.484 ^c	0.382 ^c _c	0.275 ^b	0.387 ^b	0.265 ^c

Within each column, means followed by at least one common letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

3.3. Biochemical traits: protein content, POD, and PAL activities

POD activity showed significant differences among genotypes under different stress conditions (Table 3). Under control conditions, genotype 9033 exhibited the highest POD activity, whereas genotype 2071 showed the lowest value. Following *G. tritici* inoculation, POD activity increased in several genotypes, with the highest values observed in genotypes 1528, Sardari, 2071, and Kavir. Under salinity stress, genotypes 9056 and Sardari showed relatively higher POD activities, whereas Kavir exhibited the lowest activity. Under combined salinity and *G. tritici* stress, genotypes 1528, 1642, 9033, and 9056 maintained higher POD activity compared with other genotypes (Table 3). PAL activity varied significantly among genotypes and stress treatments (Table 3). Under control conditions, genotype 1528 showed the highest PAL activity among the tested genotypes. Pathogen inoculation increased PAL activity in several genotypes, particularly 2071, 1642, Roshan, and Sardari. In contrast, salinity stress resulted in relatively lower PAL activity in some genotypes, although 9033 and 1642 maintained comparatively higher values. Under combined salinity and *G. tritici* stress, the highest PAL activities were observed in Kavir and 164 (Table 3).

Total soluble protein content differed significantly among genotypes and treatments (Table 3). Under control conditions, genotype 1528 showed the highest protein content, whereas genotype 1642 exhibited the lowest value. Following *G. tritici* inoculation, protein content increased in several genotypes, with the highest values recorded in 2071 and Roshan. Under salinity stress, genotypes 9033 and 2071 maintained relatively higher protein contents, whereas Kavir showed a lower value. Under combined salinity and pathogen stress, the highest total protein content was observed in genotype Kavir, followed by genotype 164 (Table 3).

Table 3. Effects of genotype × treatment interaction on biochemical responses (POD, PAL, and total soluble protein content) of wheat genotypes under different stress conditions.

POD										
Treatmen	Genotypes									
ts	9056	9033	2071	1642	1528	862	164	Sardari	Roshan	Kavir
Control	0.955 ^{ab}	2.156 ^a	0.219 ^d	0.755 ^c	0.896 ^b	0.600 ^b	0.437 ^c	0.810 ^b	1.142 ^{ab}	0.750 ^b
<i>G. tritici</i>	0.235 ^b	1.636 ^{ab}	1.543 ^a	0.196 ^d	2.710 ^a	0.626 ^{ab}	1.412 ^a	1.777 ^a	1.257 ^a	1.402 ^a
Salinity	1.459 ^a	0.909 ^b	1.077 ^b	1.191 ^b	0.874 ^b	0.828 ^a	0.567 ^c	1.388 ^{ab}	0.902 ^{ab}	0.141 ^c
Salinity + <i>G. tritici</i>	1.535 ^a	1.652 ^{ab}	0.725 ^c	1.403 ^a	2.352 ^a	0.336 ^c	0.983 ^b	1.135 ^{ab}	0.735 ^b	0.576 ^{bc}
PAL										
Treatmen	Genotypes									
ts	9056	9033	2071	1642	1528	862	164	Sardari	Roshan	Kavir
Control	0.026 ^a	0.025 ^b	0.023 ^c	0.0180 ^b	0.040 ^a	0.021 ^b	0.024 ^{ab}	0.023 ^{ab}	0.025 ^b	0.026 ^b
<i>G. tritici</i>	0.023 ^b	0.022 ^c	0.028 ^a	0.0253 ^a	0.025 ^b	0.025 ^a	0.023 ^{bc}	0.026 ^a	0.028 ^a	0.026 ^b
Salinity	0.025 ^a	0.028 ^a	0.025 ^b	0.0249 ^a	0.024 ^b	0.023 ^{ab}	0.021 ^c	0.022 ^{ab}	0.021 ^c	0.024 ^c
Salinity + <i>G. tritici</i>	0.022 ^b	0.022 ^c	0.024 ^{bc}	0.0245 ^a	0.022 ^c	0.023 ^{ab}	0.027 ^a	0.021 ^b	0.024 ^b	0.032 ^a
Total protein										
Treatmen	Genotypes									
ts	9056	9033	2071	1642	1528	862	164	Sardari	Roshan	Kavir
Control	147.61 ^{1a}	144.27 ^{8b}	131.500 ^c	100.38 ^{9b}	226.50 ^{0a}	117.333 ^b	135.389 ^{ab}	129.278 ^{ab}	140.944 ^b	144.83 ^{3b}
<i>G. tritici</i>	130.66 ^{7b}	126.77 ^{8c}	157.333 ^a	140.66 ^{7a}	140.11 ^{1b}	139.556 ^a	128.167 ^{bc}	145.667 ^a	157.333 ^a	147.33 ^{3b}
Salinity	142.33 ^{3a}	157.61 ^{1a}	141.500 ^b	138.72 ^{2a}	138.16 ^{7b}	129.556 ^{ab}	117.889 ^c	126.778 ^{acb}	120.944 ^c	134.44 ^{4c}
Salinity + <i>G. tritici</i>	124.55 ^{6b}	124.27 ^{8c}	132.278 ^{bc}	136.22 ^{2a}	123.72 ^{2c}	132.611 ^{ab}	151.500 ^a	118.444 ^b	134.278 ^b	179 ^a

Within each column, means followed by at least one common letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

3.4. Principal component analysis (PCA)

3.4.1. Control environment

Under control conditions, PCA extracted two principal components that together explained 78.0% of the total variability among measured traits (Table 4). PC1 accounted for 49.2% of the total variability and showed strong positive loadings for total protein content (0.632) and PAL activity (0.632), indicating their major contribution to this component. PC2 explained 28.9% of the variability and was mainly associated with root K^+/Na^+ ratio (0.616) and POD activity (0.677), whereas shoot K^+/Na^+ ratio showed a negative loading (-0.392).

The genotype–trait biplot (Figures 1 and 2) showed that genotypes Kavir, Roshan, 9056, and 9033 were positioned in the positive region of both PC1 and PC2, indicating their association with traits contributing positively to these components. In contrast, genotypes Sardari, 2071, and 164 were located in the negative region of both components. Genotype 1642 was positioned in the upper-left quadrant and showed positive association mainly with POD activity and root K^+/Na^+ ratio, whereas its association with total protein content and PAL activity was weaker compared with genotypes located in the upper-right quadrant.

Table 4. Principal component analysis (PCA) loadings, eigenvalues, and explained variance of physiological and biochemical traits under control conditions.

Variable	PC1	PC2
K/Na in Root	-0.394	0.616
K/Na in shoot	-0.049	-0.392
POD	0.211	0.677
Total Protein	0.632	0.064
PAL	0.632	0.064
Eigenvalue	2.4578	1.4433
Proportion	0.492	0.289
Cumulative	0.492	0.780

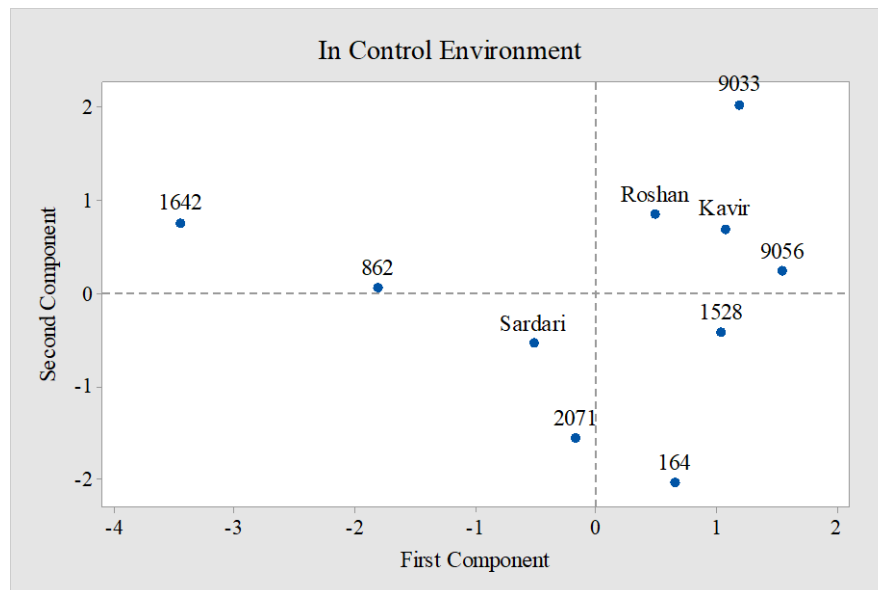


Figure 1. PCA score plot showing the distribution of wheat genotypes under control conditions.

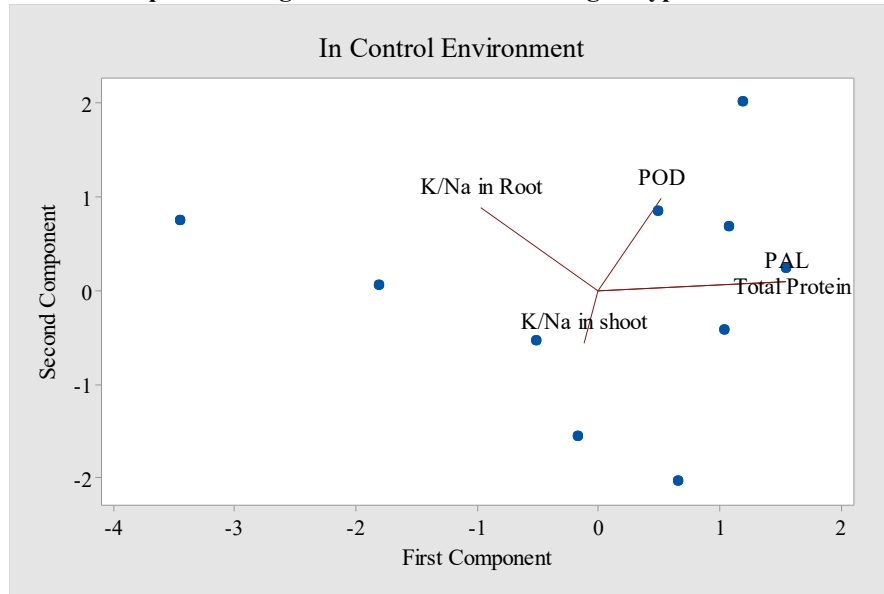


Figure 2. PCA biplot showing relationships among genotypes and physiological traits under control conditions.

3.4.2. PCA Under Salinity Stress

Under salinity stress, PCA revealed that the first two principal components explained 80.4% of the total variability among measured traits, with PC1 and PC2 accounting for 52.0% and 28.4% of the variation, respectively (Table 5). Therefore, these two components provided an adequate representation of the relationships among the evaluated physiological and biochemical traits.

PC1 showed strong positive loadings for total protein content (0.609) and PAL activity (0.609), whereas root K^+/Na^+ ratio (-0.403) and shoot K^+/Na^+ ratio (-0.240) showed negative loadings. These loading patterns indicate that PC1 mainly reflected variation associated with protein accumulation and PAL activity in relation to ionic traits under salinity stress. Genotypes located on the positive side of PC1 were more closely associated with higher values of protein content and PAL activity, whereas genotypes with negative PC1 scores showed stronger associations with K^+/Na^+ ratios.

PC2 was mainly associated with shoot K^+/Na^+ ratio (-0.717) and POD activity (-0.545), whereas root K^+/Na^+ ratio showed a positive loading (0.417). This pattern suggests that PC2 was mainly related to the relationship between shoot ionic status and POD activity. Genotypes with positive PC2 scores showed stronger associations with root K^+/Na^+ ratio, whereas negative PC2 scores were associated with shoot K^+/Na^+ ratio and POD activity (Figures 3 and 4).

Overall, PCA demonstrated that genotypic variation under salinity stress was mainly explained by differences in protein content, PAL activity, and ionic traits. The distribution of genotypes along PC1 and PC2 indicated distinct patterns of association among physiological and biochemical responses under saline conditions.

Table 5- Factor loadings, eigenvalues, and explained variance of the first two principal components under salinity stress conditions

Variable	PC1	PC2
K/Na in Root	-0.403	0.417
K/Na in shoot	-0.240	-0.717
POD	0.198	-0.545
Total Protein	0.609	0.085
PAL	0.609	0.085
Eigenvalue	2.6019	1.4185
Proportion	0.520	0.284
Cumulative	0.520	0.804

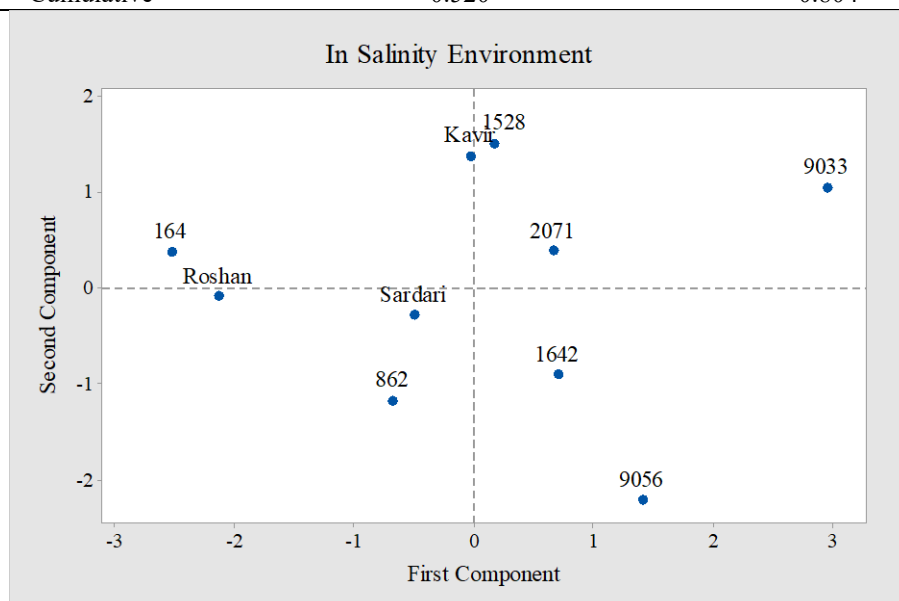


Figure 3. Principal component analysis (PCA) score plot illustrating the distribution of wheat genotypes based on physiological traits under salinity stress.

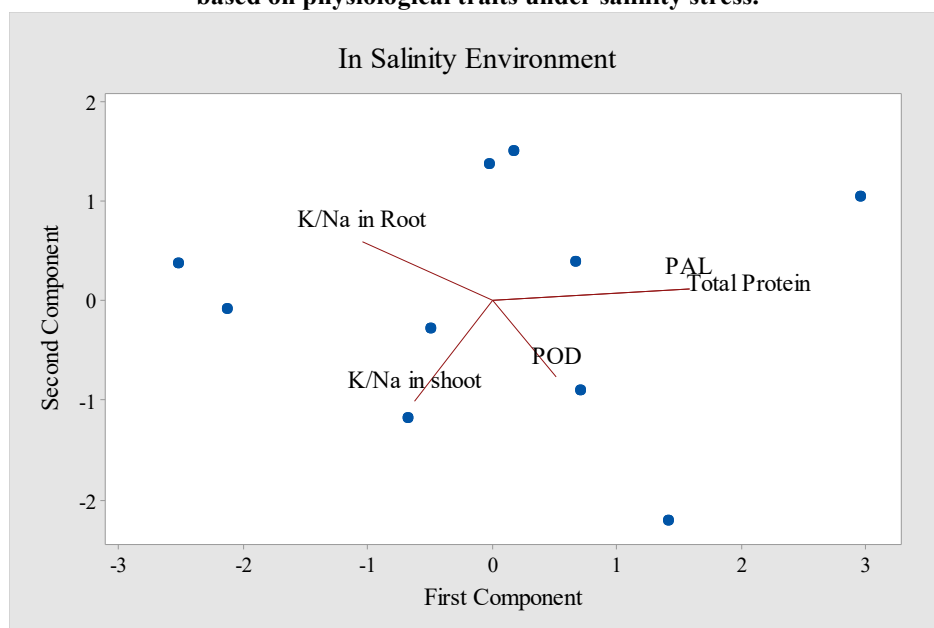


Figure 4. PCA loading plot showing the contribution of physiological traits to the first two principal components under salinity stress.

3.4.3. PCA Under Pathogen Inoculation

Under pathogen inoculation, PCA revealed that the first two principal components explained 81.4% of the total variability among the measured traits, with PC1 and PC2 accounting for 56.0% and 25.4% of the variation, respectively (Table 6). These two components therefore provided an adequate representation of trait relationships and genotype distribution under *G. tritici* infection.

The first component (PC1; eigenvalue = 2.80) accounted for 56.0% of the total variation and was mainly associated with biochemical traits in relation to ionic characteristics. Total protein and PAL activity showed the highest positive loadings on PC1 (0.567), while root and shoot K^+/Na^+ ratios showed negative loadings (−0.221 and −0.298, respectively). Genotypes located on the positive side of PC1, including 9033, 9056, and Sardari (Figure 5), showed stronger associations with total protein and PAL activity, whereas genotypes positioned on the negative side were more closely associated with higher K^+/Na^+ ratios.

PC2 (eigenvalue = 1.27) explained 25.4% of the total variation. Root K^+/Na^+ ratio showed the strongest negative loading (−0.745), followed by shoot K^+/Na^+ ratio (−0.531) and POD activity (−0.534), whereas total protein and PAL activity contributed weakly to this component (−0.138). This loading pattern indicates that PC2 was mainly related to variation in ionic traits and POD activity among genotypes under pathogen infection.

The genotype–trait biplot (Figures 5 and 6) showed clear separation among genotypes under *G. tritici* inoculation. Genotypes 1528, 862, 2071, and Roshan were positioned in the negative region of PC1 and PC2 and showed stronger associations with K^+/Na^+ ratios and POD activity. In contrast, genotypes 9033, 9056, Sardari, and Kavir were mainly distributed on the positive side of PC1, showing stronger associations with biochemical traits, particularly PAL and total protein content.

Table 6- Eigenvalues, explained variance, and factor loadings of physiological traits for the first two principal components under *Gaeumannomyces tritici* infection.

Variable	PC1	PC2
K/Na in Root	-0.221	-0.745
K/Na in shoot	-0.298	-0.531

POD	0.469	-0.534
Total Protein	0.567	-0.138
PAL	0.567	-0.138
Eigenvalue	2.8001	1.2692
Proportion	0.560	0.254
Cumulative	0.560	0.814

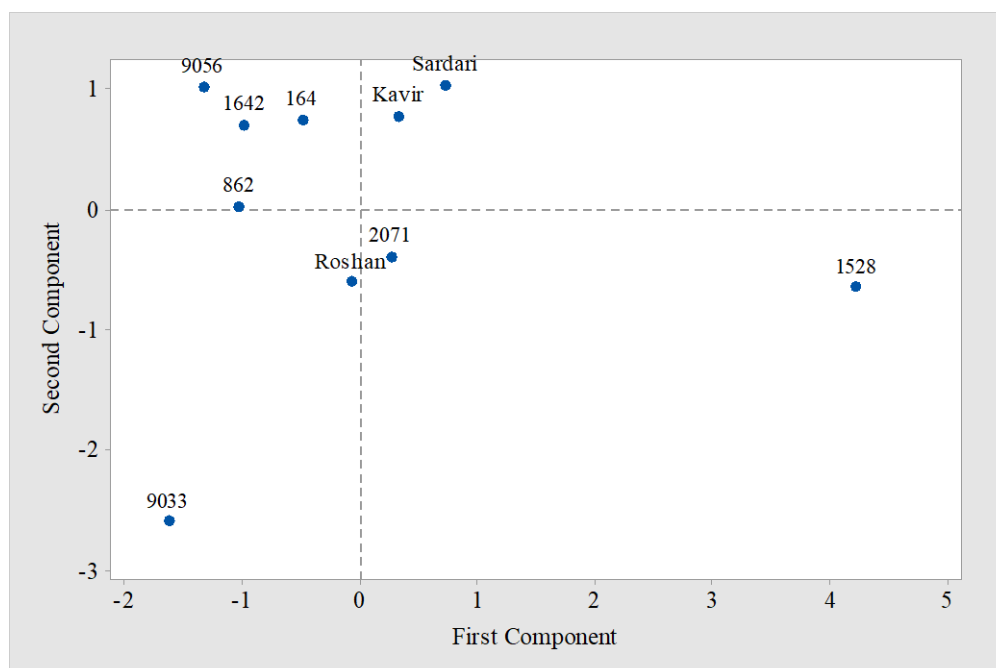


Figure 5- PCA biplot showing genotype distribution under *Gaeumannomyces tritici* infection.

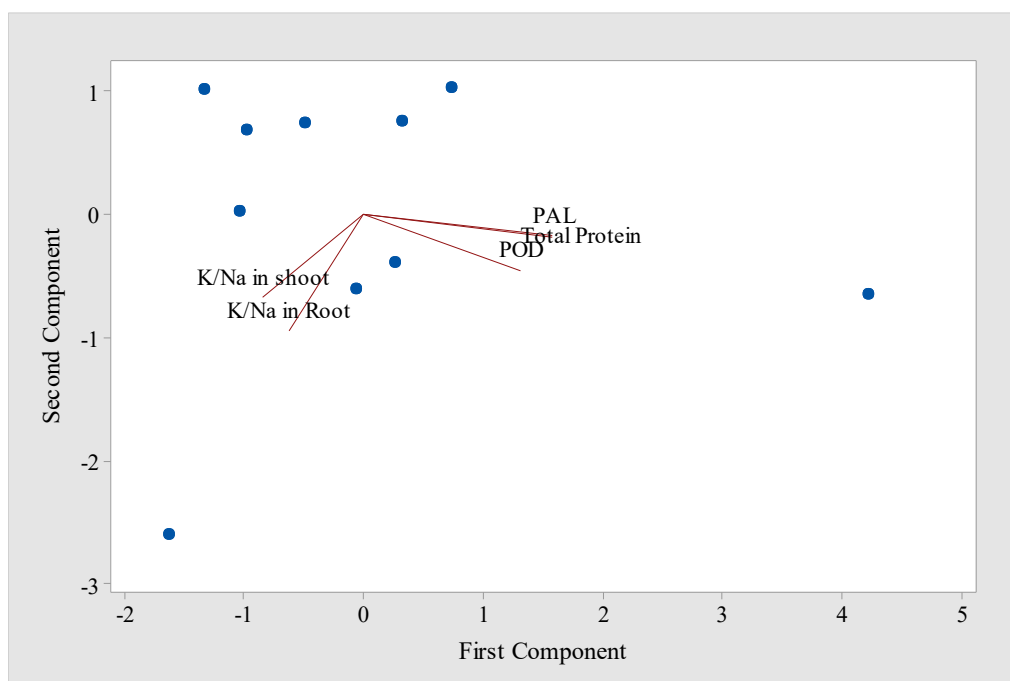


Figure 6- PCA loading plot illustrating the contribution of physiological traits to principal components under *Gaeumannomyces tritici* infection.

3.4.4. PCA Under Combined Salinity and disease stress

Under combined salinity and disease stress, PCA revealed that the first two principal components explained 81.8% of the total variability among measured traits, with PC1 and PC2 accounting for 54.1% and 27.7% of the variation, respectively (Table 7). These two components provided an adequate representation of trait relationships and genotype distribution under combined stress conditions.

The first component (PC1; eigenvalue = 2.70) accounted for 54.1% of the total variation and mainly reflected the relationship between biochemical traits and shoot ionic/oxidative parameters. Total protein and PAL activity showed high positive loadings on PC1 (both 0.585), whereas shoot K^+/Na^+ ratio (-0.393) and POD activity (-0.385) showed negative loadings (Figures 7 and 8). Genotypes located on the positive side of PC1 were more strongly associated with total protein and PAL activity, whereas those positioned on the negative side showed stronger associations with shoot K^+/Na^+ ratio and POD activity.

PC2 (eigenvalue = 1.38) explained 27.7% of the total variation and was mainly associated with root ionic status and POD activity. Root K^+/Na^+ ratio showed the highest positive loading (0.800), followed by POD activity (0.549), while other traits contributed weakly to this component. Therefore, positive PC2 scores were mainly associated with higher root K^+/Na^+ ratio and POD activity, whereas negative PC2 scores represented the opposite association pattern.

The genotype biplot (Figure 7) showed clear separation among genotypes under combined salinity and disease stress. Genotypes located in the negative PC1 and positive PC2 quadrant, including 1528 and 1642, were closely associated with higher root K^+/Na^+ ratio and POD activity, together with relatively higher shoot ionic status. In contrast, genotypes positioned on the positive side of PC1 or negative side of PC2 showed stronger associations with biochemical traits or weaker ionic and oxidative-related responses.

Overall, PCA indicated that genotype differentiation under combined stress was mainly driven by ionic traits, POD activity, and biochemical parameters, reflecting distinct physiological response patterns among wheat genotypes under simultaneous salinity and pathogen stress.

Table 7. Eigenvalues, explained variance, and trait loadings of the first two principal components under combined salinity and disease stress

Variable	PC1	PC2
K/Na in Root	0.116	0.800
K/Na in shoot	-0.393	0.131
POD	-0.385	0.549
Total Protein	0.585	0.146
PAL	0.585	0.146
Eigenvalue	2.7055	1.3852
Proportion	0.541	0.277
Cumulative	0.541	0.818

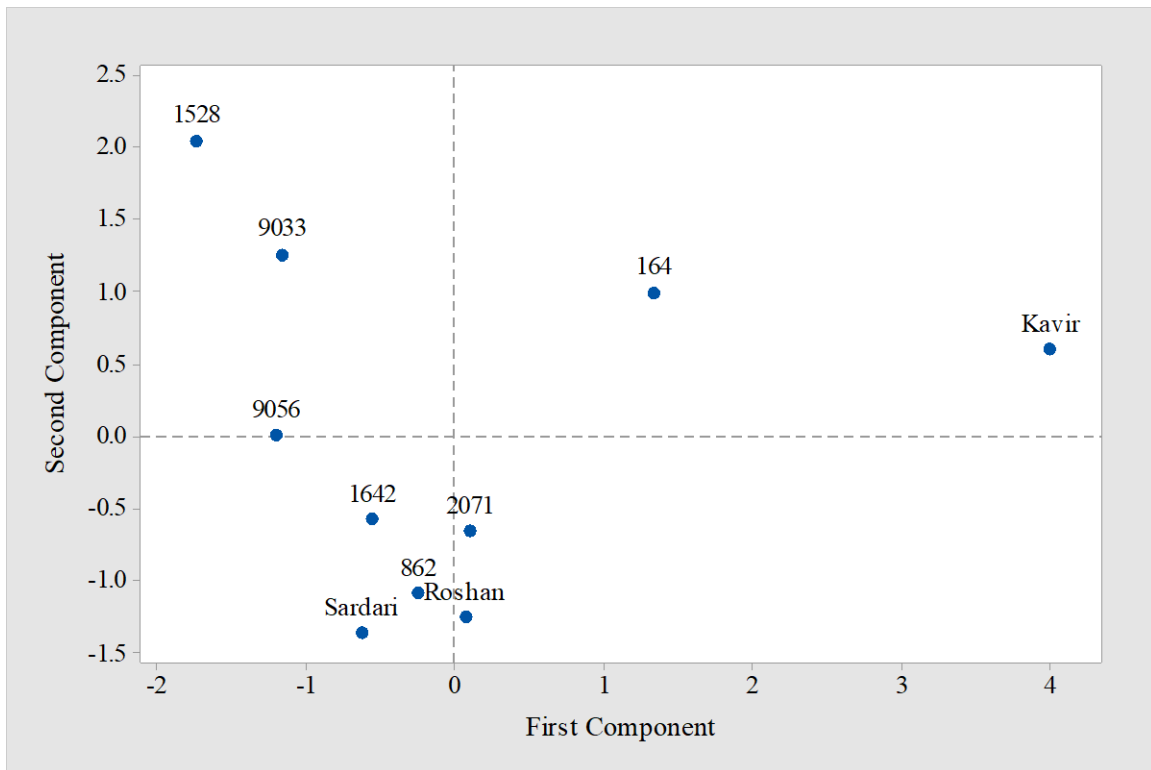


Figure 7- PCA biplot showing genotype distribution and trait relationships under combined salinity and *Gaeumannomyces tritici* stress.

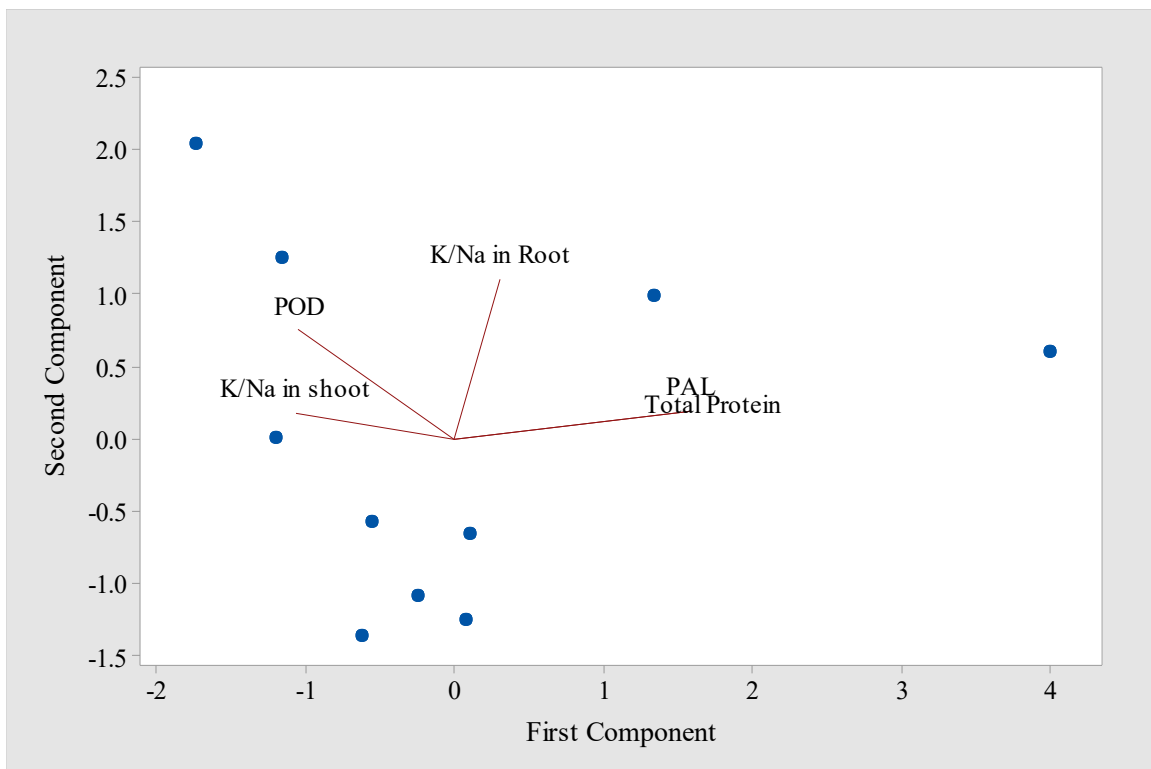


Figure 8- PCA loading plot illustrating the contribution of physiological and biochemical traits under combined salinity and *Gaeumannomyces tritici* stress.

3.5. Cluster Analysis

The results of cluster analysis performed using Ward's method based on squared Euclidean distance separated the 30 genotype–environment combinations into two distinct clusters with contrasting physiological response patterns (Figure 9). Cluster 1 comprised 19 observations, whereas Cluster 2 included 11 observations, indicating the existence of two major physiological strategies associated with wheat responses to salinity and pathogen stresses. This classification was consistent with the patterns observed in PCA analysis.

Based on the standardized trait means presented in Table 8, Cluster 1 exhibited positive values for root K^+/Na^+ ratio (+0.657), shoot K^+/Na^+ ratio (+0.894), and POD activity (+0.395), while showing negative values for total protein (−0.558) and PAL activity (−0.558). In contrast, Cluster 2 displayed an opposite pattern, characterized by lower ionic indices and POD activity but higher total protein and PAL values. Therefore, Cluster 1 can be described as representing an ionic–antioxidant regulation pattern, whereas Cluster 2 represents a protein–phenylpropanoid defense-associated pattern.

The distribution of genotype–environment combinations within clusters further demonstrated this physiological differentiation. Cluster 1 mainly consisted of combinations exposed to salinity alone or combined salinity–pathogen stress, including 1528 (salinity + *G. tritici*), 9033 (salinity + *G. tritici*), 1642 (salinity + *G. tritici*), and Sardari (salinity). The predominance of saline treatments in this cluster suggests that under salt exposure, several wheat genotypes-maintained stress adaptations primarily through improved ionic balance and antioxidant capacity, as reflected by higher K^+/Na^+ ratios and POD activity.

Conversely, Cluster 2 contained a greater proportion of pathogen-inoculated combinations, including Kavir (*G. tritici*), Sardari (*G. tritici*), 2071 (*G. tritici*), and 862 (*G. tritici*). These combinations were characterized by higher PAL activity and total protein content, indicating stronger activation of phenylpropanoid-associated and protein-based defense responses following pathogen challenge. The presence of some salinity and combined stress treatments within this cluster, such as Kavir under salinity and 2071 under combined stress, indicates that different genotypes may employ distinct physiological strategies depending on stress type and genetic background (Table 8).

Overall, cluster analysis supports the PCA findings and demonstrates that wheat genotypes differ in their physiological strategies under single and combined stresses. Some genotypes primarily maintain ionic homeostasis and antioxidant activity, whereas others rely more strongly on metabolic defense-related responses.

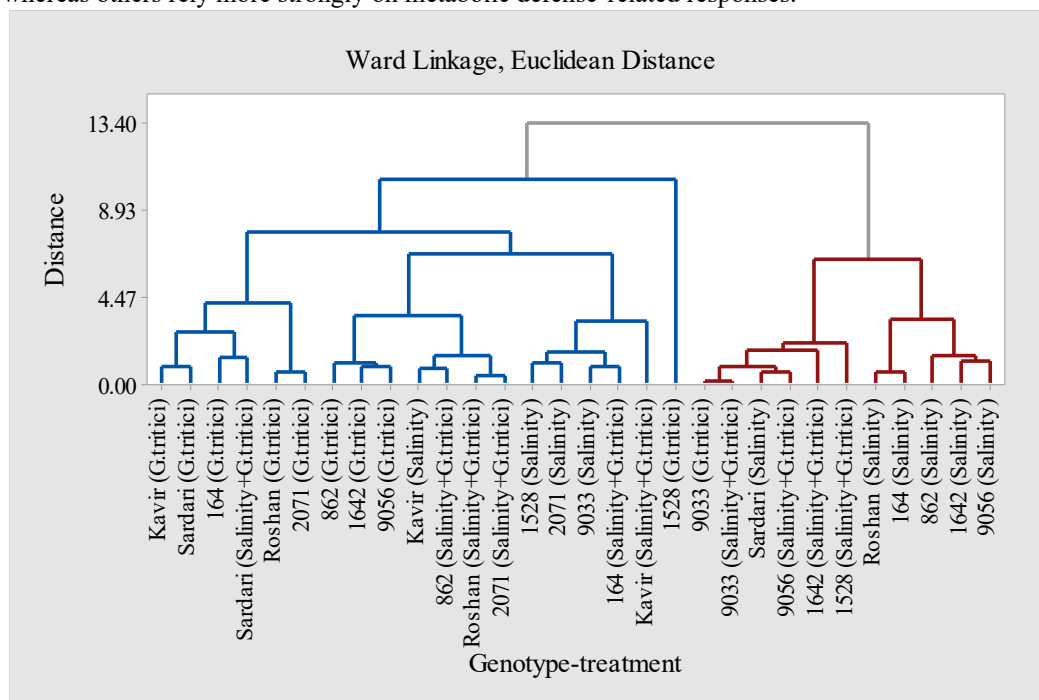


Figure 9- Dendrogram of 30 genotype–environment combinations generated by Ward’s clustering method based on standardized physiological traits

Table 8- Cluster characteristics, standardized trait means, and genotype–environment combinations identified by Ward’s clustering method based on physiological and biochemical traits.

Traits	Cluster1	Cluster2
K/Na in Root	0.657334	-0.380562
K/Na in shoot	0.893997	-0.517577
POD	0.394662	-0.228489
Total Protein	-0.558124	0.323124
PAL	-0.558129	0.323127
Number of observations	19	11
Within cluster sum of squares	90.3449	19.7431
Average distance from centroid	1.89413	1.28335
Maximum distance from centroid	6.14901	1.82489
Genotype-Environment	Kavir (<i>G.tritici</i>), Sardari (<i>G.tritici</i>) 164 (<i>G. tritici</i>), Sardari (Salinity + <i>G. tritici</i>), Roshan (<i>G. tritici</i>), 2071 (<i>G. tritici</i>), 862 (<i>G.tritici</i>), 164 (<i>G. tritici</i>), 9056 (<i>G.tritici</i>), Kavir (Salinity), 862 (Salinity + <i>G. tritici</i>), Roshan (Salinity + <i>G.tritici</i>), 2071 (Salinity + <i>G.tritici</i>), 1528 (Salinity), 2071 (Salinity), 9033 (Salinity), 164 (Salinity + <i>G.tritici</i>), Kavir (Salinity + <i>G.tritici</i>), 1528 (<i>G.tritici</i>)	9033 (<i>G.tritici</i>), 9033 (Salinity + <i>G.tritici</i>), Sardari (Salinity), 9056 (Salinity + <i>G.tritici</i>), 1642 (Salinity + <i>G.tritici</i>), 1528 (Salinity + <i>G.tritici</i>), Roshan (Salinity, 164 (Salinity), 862 (Salinity), 1642 (Salinity), 9056 (Salinity)

The analysis of variance between clusters (Table 9) further supported the physiological separation identified by cluster analysis. The cluster effect was significant at the 1% probability level ($p < 0.01$) for all evaluated traits except POD activity, which did not show significant differences between clusters. These results confirm that the two clusters were significantly differentiated by root and shoot K^+/Na^+ ratios, total protein content, and PAL activity. Cluster 1 exhibited greater within-cluster variability than Cluster 2, as indicated by its higher average and maximum distances from the centroid. This greater dispersion may be associated with the broader range of genotype–environment combinations represented within this cluster, including salinity and combined stress conditions (Table 9).

Table 9- Analysis of variance for the studied traits between clusters derived from cluster analysis.

S.O.V	df	MS				
		K/Na in root	K/Na in shoot	POD	Total Protein	PAL
Group	1	0.0213**	0.1868**	0.9750 ^{ns}	2439.3**	0.000079**
Error	28	0.0021	0.0072	0.3385	379.9	0.000012
Total	29					

3.5.1. Mean comparisons of clusters obtained from cluster analysis

Comparison of mean shoot K^+/Na^+ ratios between clusters (Figure 10) showed that Cluster 1 exhibited a significantly higher ratio than Cluster 2. This difference indicates that genotype–environment combinations grouped in Cluster 1 maintained a more favorable shoot ionic balance under stress conditions compared with those in Cluster 2. The higher shoot K^+/Na^+ ratio observed in Cluster 1 suggests improved maintenance of potassium–sodium homeostasis, which is considered an important component of salt stress adaptation.

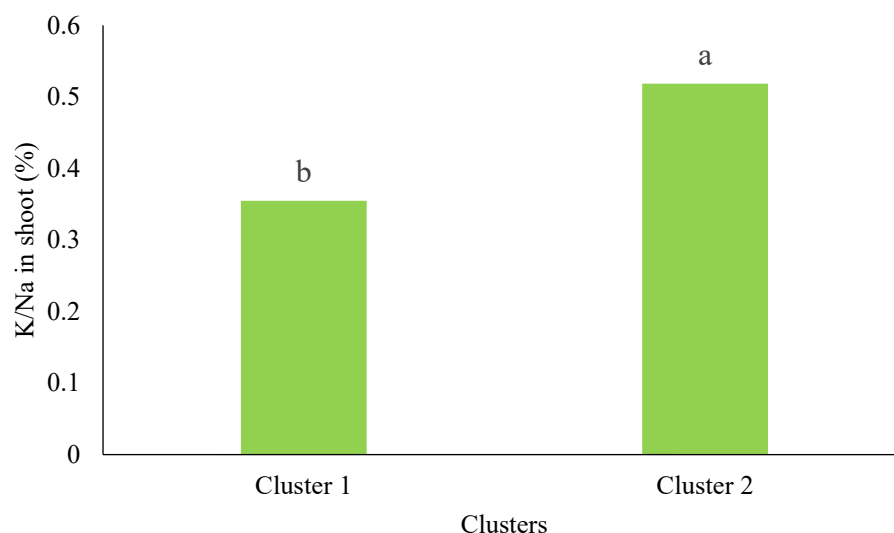


Figure 10- Mean shoot K^+/Na^+ ratios of the two clusters identified by Ward's method. Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

A similar pattern was observed for root K^+/Na^+ ratios (Figure 11), where Cluster 1 showed a significantly higher mean value than Cluster 2. This indicates that the ionic response pattern distinguishing Cluster 1 was evident at both root and shoot levels. Higher root K^+/Na^+ ratios may reflect a greater ability to maintain ionic homeostasis under stressful conditions, whereas the lower values observed in Cluster 2 indicate a weaker ionic regulation capacity. Together, these results suggest that root and shoot ionic traits contributed substantially to the separation of the two physiological strategies identified by cluster analysis.

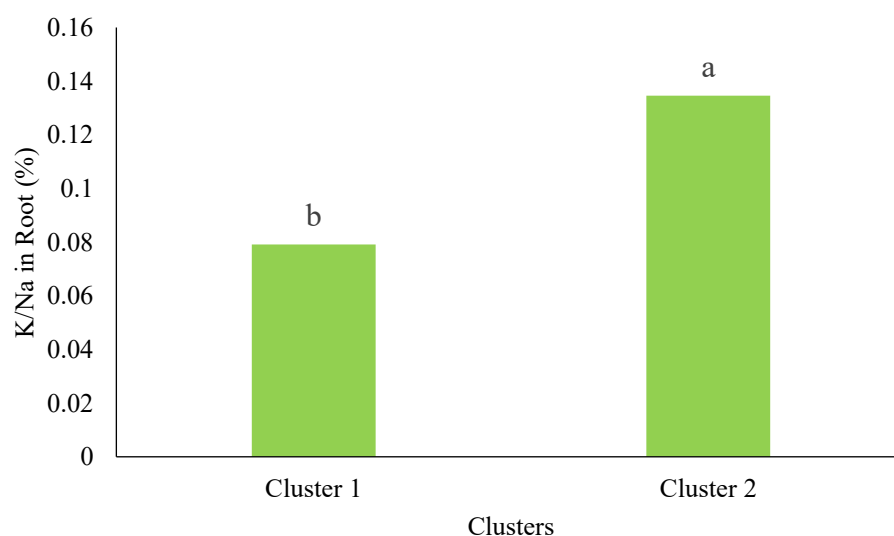


Figure 11- Mean root K^+/Na^+ ratios of the two clusters identified by Ward's method. Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

In contrast to ionic traits, total protein content showed an opposite pattern (Figure 12). Cluster 2 exhibited a significantly higher mean protein content than Cluster 1. The increased protein accumulation in Cluster 2, which included a high proportion of pathogen-inoculated combinations, suggests enhanced activation of protein-related stress responses following fungal challenge. Conversely, the lower protein content observed in Cluster 1, which was mainly associated with saline treatments, may indicate a different stress adaptation pattern in which resources are

preferentially allocated toward maintaining ionic balance and osmotic adjustment rather than increasing soluble protein accumulation.

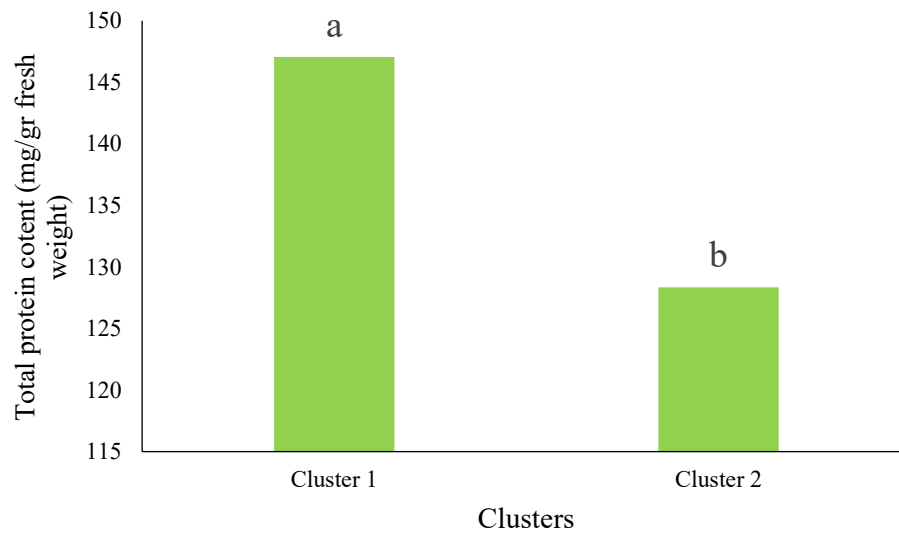


Figure 12- Mean total protein content of the two clusters identified by Ward's method. Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

A comparable trend was observed for PAL activity (Figure 13), with Cluster 2 showing significantly higher PAL activity than Cluster 1. Since PAL represents a key enzyme involved in the phenylpropanoid pathway, its higher activity in Cluster 2 indicates stronger activation of phenylpropanoid-associated responses, particularly among genotype–environment combinations exposed to pathogen infection without salinity. In contrast, the lower PAL activity in Cluster 1, which was dominated by saline treatments, suggests that these combinations relied less on phenylpropanoid activation and were primarily characterized by stronger ionic regulation patterns.

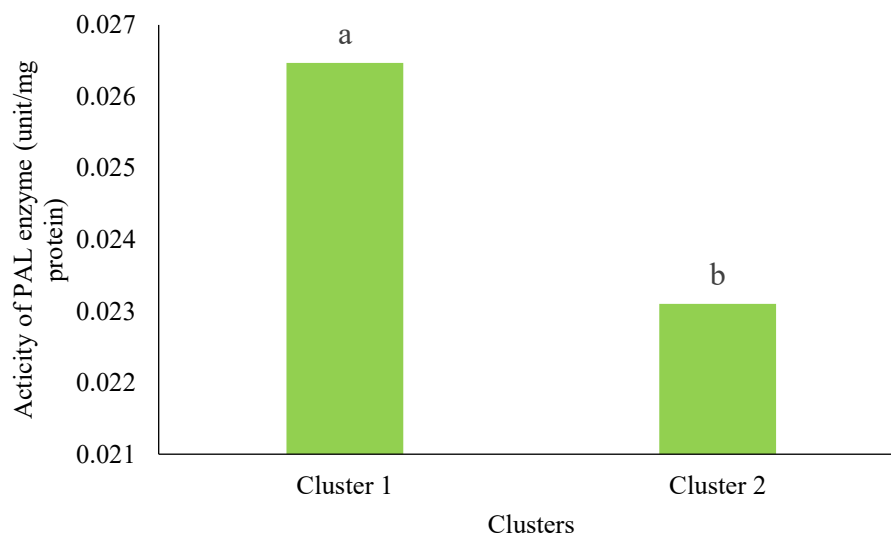


Figure 13- Mean PAL activity of the two clusters identified by Ward's method. Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

4. Discussion

The analysis of variance revealed significant differences among the studied genotypes for all evaluated traits, including total protein content, POD and PAL activities, and K^+/Na^+ ratios in both roots and shoots. This finding is consistent with previous studies reporting substantial genetic diversity among wheat genotypes in their physiological responses to salt stress [40–43]. Furthermore, the significant genotype $\times$ treatment interaction indicates that genotypic responses were highly dependent on stress type, with each genotype exhibiting distinct physiological and biochemical adjustment patterns. This highlights the importance of evaluating genotype performance under combined stress conditions rather than relying solely on responses observed under individual stresses.

Under control conditions, genotypes 1642 and 1528 displayed the highest root K^+/Na^+ ratios, reflecting a superior basal capacity for maintaining ionic homeostasis, whereas genotypes 164 and 2071 exhibited lower ratios. Such differences under non-stress conditions may represent inherent genetic variation in ion regulation mechanisms. Following infection with *G. tritici*, the root K^+/Na^+ ratio declined markedly in most genotypes, suggesting that pathogen infection negatively affected root ion uptake and transport processes. This reduction was particularly evident in genotypes previously classified as susceptible, such as Roshan and 9033. *G. tritici* colonization of root and crown tissues can impair root functionality and interfere with water and nutrient acquisition [44], which may contribute to the disruption of K^+/Na^+ balance observed under pathogen stress.

Under salinity alone, genotypes 1528 and 164 maintained higher root K^+/Na^+ ratios, suggesting a greater capacity for maintaining ionic homeostasis under salt stress. This finding is consistent with Cuin et al. (2008), who demonstrated that maintenance of a high K^+/Na^+ ratio is an important physiological component of salt tolerance [9]. The ability to sustain a favorable K^+/Na^+ balance under salinity is associated with coordinated regulation of membrane transport systems, including HKT transporters, which contribute to restricting Na^+ accumulation and maintaining cellular ion homeostasis [45]. Under combined salinity and disease stress, although most genotypes showed a relative decline, genotypes 1528 and Kavir maintained comparatively stable K^+/Na^+ ratios, indicating better preservation of ionic homeostasis under concurrent stress. This suggests that these genotypes possess physiological traits associated with improved adaptation to concurrent stresses and may represent valuable genetic resources for breeding programs. Under such combined stress, ionic regulation appears to represent a major component of plant adjustment, as indicated by the strong contribution of K^+/Na^+ ratios to genotype discrimination in the present study. This finding agrees with the concept that plants integrate multiple stress-responsive mechanisms when exposed to simultaneous abiotic and biotic challenges [22].

A similar pattern was observed for shoot K^+/Na^+ ratios. Under control conditions, genotypes Sardari and 164 exhibited the highest shoot ratios, indicating a greater capacity for maintaining K^+/Na^+ balance in aerial tissues under non-stress conditions. Under salinity stress, genotypes 862 and 1528 maintained relatively high shoot K^+/Na^+ ratios, consistent with Shahzad et al. (2022), who identified salt-tolerant rice genotypes based on their ability to maintain higher shoot K^+/Na^+ ratios under saline conditions [46]. Under combined salinity and disease stress, genotypes 1528 and 164 remained superior in maintaining shoot ionic homeostasis. This consistent advantage highlights shoot K^+/Na^+ ratio as an important physiological trait for evaluating wheat performance and identifying superior genotypes under saline and combined-stress environments.

POD activity varied significantly among genotypes and treatments. Under control conditions, genotype 9033 exhibited the highest activity, whereas 2071 showed the lowest value, indicating substantial genetic variation in basal antioxidant capacity. Following pathogen inoculation, POD activity increased markedly in genotypes 1528 and Sardari, suggesting the involvement of this enzyme in oxidative defense responses triggered by *G. tritici* infection. Under salinity stress, genotypes 9056 and Roshan displayed higher POD activities, whereas Kavir showed a pronounced reduction. Enhanced antioxidant enzyme activity under salt stress has previously been reported as an important component of stress tolerance mechanisms in plants [47]. Under combined salinity and disease stress, genotypes 1528 and 1642 maintained relatively high POD activities, suggesting a stronger capacity for activating antioxidant responses under simultaneous stress conditions. However, the cluster analysis revealed that the group effect for POD activity was not significant, indicating that although POD contributes to stress responses, its variability among genotype–environment combinations limits its reliability as a single discriminating trait compared with ionic parameters such as K^+/Na^+ ratio.

Under control conditions, genotype 1528 exhibited the highest PAL activity, suggesting a greater basal capacity for activation of the phenylpropanoid pathway. Pathogen infection resulted in a marked increase in PAL activity, particularly in genotypes 2071 and Roshan, indicating activation of secondary metabolic defense responses following *G. tritici* challenge. PAL is a key entry-point enzyme in the phenylpropanoid pathway, regulating the synthesis of

phenolic compounds, phytoalexins, and lignin, and its induction during pathogen attack has been widely documented [48]. In contrast, salinity stress generally reduced PAL activity, although genotypes 9033 and 1642 maintained comparatively higher levels. This suppression was also observed under combined salinity and disease stress, suggesting that under saline conditions plants may prioritize energy allocation toward ion homeostasis and osmotic adjustment rather than costly secondary metabolic defense pathways.

The suppression of PAL activity under salinity stress can be attributed to several interconnected physiological and molecular mechanisms. First, as proposed by Wu et al. (2025), PAL functions as a central “metabolic valve” that regulates carbon flux allocation between primary metabolism and the phenylpropanoid pathway. Under salinity stress, this metabolic regulation may shift carbon flux toward essential primary metabolic processes, including the synthesis of compatible osmolytes such as proline and soluble sugars, as well as mechanisms associated with ionic regulation, while reducing resource allocation to energetically costly secondary metabolic defense pathways [19].

Second, Singh et al. (2025) demonstrated that the expression of TaPAL genes in wheat is significantly regulated under salt stress, with multiple stress-responsive cis-regulatory elements present in their promoter regions. This finding suggests that PAL suppression under salinity is not merely a passive consequence of stress-induced metabolic disruption but may represent a genetically regulated adaptive response [43].

Third, Dunn et al. (1998) reported a negative correlation between PAL activity and salinity intensity in citrus, showing that increased salt stress reduced PAL activity while promoting arginine accumulation. These findings suggest that under saline conditions, nitrogen metabolism may be preferentially redirected toward amino acid synthesis and osmotic adjustment rather than secondary metabolite production [21].

Fourth, salinity stress induces ABA accumulation, which can interact antagonistically with SA-mediated defense signaling and potentially suppress the expression of phenylpropanoid pathway genes, including PAL [30, 31].

Fifth, because activation of the phenylpropanoid pathway requires considerable metabolic investment, reduced PAL activity under salinity may represent an adaptive strategy that conserves cellular resources and redirects energy toward ion homeostasis and osmotic adjustment, which are critical for plant survival under salt stress [19, 20].

Nevertheless, under combined stress, genotypes 1528 and Kavir displayed the highest PAL activities, suggesting their greater activation of phenylpropanoid-related defense responses under concurrent stress conditions and their potential ability to balance defense activation with ionic regulation.

Significant genotypic differences were observed for total protein content. Under control conditions, the highest protein level was recorded in genotype 1528. Pathogen challenge with *G. tritici* resulted in a notable increase in protein content, particularly in genotypes 1528 and Roshan, suggesting the induction of defense-related metabolic responses. This increase may be associated with enhanced synthesis of pathogenesis-related (PR) proteins and defense-associated enzymes during pathogen challenge [49]. Under salinity stress, genotypes 9033 and 2071 maintained relatively elevated protein levels, possibly due to increased accumulation of heat shock proteins (HSPs) and other protective proteins involved in osmotic stress adaptation [50].

The increase in total protein content under salinity stress in certain genotypes can be explained by several interconnected mechanisms. First, salinity induces the expression of genes encoding HSPs and other molecular chaperones that contribute to protein stabilization and prevention of stress-induced denaturation [50].

Second, salt stress enhances the production of antioxidant enzymes, including SOD, CAT, APX, and POD; because these enzymes are proteins, their accumulation can contribute to increased total soluble protein content [42, 47]. Third, salinity can stimulate the expression of ion transporter proteins, including HKT and SOS1, which play essential roles in maintaining cellular ion homeostasis [9, 45]. Fourth, Bhutta (2011) demonstrated that increased protein accumulation under stress may reflect active de novo protein synthesis rather than simply the accumulation of free amino acids [41]. Therefore, the elevated protein levels observed in genotypes 9033 and 2071 under salinity suggest enhanced activation of protein-based protective mechanisms, although these genotypes were less efficient in maintaining ionic balance compared with genotypes 1528 and 1642.

Under combined salinity and disease stress, genotype Kavir exhibited the highest total protein content, indicating strong activation of protein-associated responses under concurrent stress conditions. However, compared with pathogen infection alone, total protein appeared to have a weaker discriminatory role under combined stress, suggesting that when salinity is superimposed on pathogen pressure, ionic regulation becomes a more dominant physiological determinant than protein-based defense responses.

PCA across the four environments revealed a consistent pattern of stress-dependent trait associations. Under control conditions, PC1 represented a metabolic-defense axis mainly associated with total protein and PAL, whereas PC2

primarily reflected variation in root ionic status and POD activity. Genotypes Kavir, Roshan, 9056, and particularly 9033, clustered in the positive quadrant of both components, indicating higher values for these metabolic and oxidative-related traits under non-stress conditions.

Under salinity stress, PC1 represented a contrast between metabolic-defense capacity and ionic regulation, with genotypes positioned on the positive side showing higher total protein and PAL values but lower K^+/Na^+ ratios. PC2 reflected the relationship between shoot ionic status and oxidative responses. These results indicate that under saline conditions, wheat genotypes employ different strategies, balancing investment in protein-based defense mechanisms against the maintenance of ionic homeostasis.

Under pathogen infection, PC1 contrasted metabolic-defense traits with ionic characteristics. Genotypes located in the negative quadrant of both components, including 1528, 862, 2071, and to some extent Roshan, exhibited higher root and shoot K^+/Na^+ ratios together with enhanced POD activity, suggesting a favorable physiological response pattern under *G. tritici* challenge. Conversely, genotypes such as 9033 and 9056, positioned on the positive side of PC1, showed stronger association with PAL and total protein accumulation, indicating greater activation of metabolic defense pathways.

Under combined salinity and disease stress, an even clearer physiological separation was observed. PC1 represented a contrast between metabolic-protein defense and shoot ionic/oxidative traits, whereas PC2 was mainly associated with root ionic status and POD activity. Genotypes positioned in the negative PC1 and positive PC2 quadrant, including 1528 and 1642 and possibly 2071 and 862, combined higher shoot K^+/Na^+ ratios, favorable root ionic balance, and enhanced POD activity. This combination suggests that these genotypes possess a favorable physiological strategy for coping with simultaneous salinity and pathogen stresses.

Cluster analysis further corroborated these findings. At a Euclidean distance of 10.5272, the 30 genotype–environment combinations were divided into two distinct clusters. Cluster 1 (19 observations) mainly comprised salinity and combined stress treatments and adopted an "ionic–antioxidant regulation" strategy (positive standardized means for root/shoot K^+/Na^+ and POD; negative for total protein and PAL). Cluster 2 (11 observations) mainly included disease treatments without salinity and followed a "secondary metabolic defense" strategy (positive for total protein and PAL; negative for ionic indices). This dual separation clearly demonstrates the existence of two opposing and switchable strategies in response to environmental stresses.

Based on these findings, we propose a conceptual model for wheat responses to salinity and disease stresses:

In the absence of salinity (control or disease alone), the plant adopts a secondary metabolic defense strategy. The PAL pathway is activated, phenolic compounds and phytoalexins are synthesized, and defense proteins accumulate. This strategy is effective against pathogens but carries a high energy cost [19, 20].

In the presence of salinity (salinity alone or combined stress), the plant switches to an ionic–antioxidant regulation strategy. It prioritizes maintaining the K^+/Na^+ balance in roots and shoots and strengthens the antioxidant system (POD), while suppressing or marginalizing costly pathways such as PAL. Salinity acts as the dominant stressor [21, 51].

Several factors explain why plants suppress PAL and prioritize ionic regulation under salinity. First, the phenylpropanoid pathway is energetically expensive, while ionic regulation—mediated by transporters such as HKT and SOS1—provides a more rapid and energy-efficient response to salinity stress [19, 20]; [9, 45]. Second, the primary threat under salinity is Na^+ toxicity and K^+ deficiency [5]; therefore, plant survival depends more on ion homeostasis than on secondary metabolite production. Third, ABA accumulation under salinity suppresses SA signaling and reduces the expression of phenylpropanoid pathway genes, including PAL [30, 31]. Fourth, Na^+ accumulation may directly inhibit PAL enzyme activity or suppress PAL gene expression through ABA-dependent signaling pathways [21].

Superior genotypes such as 1528 and 1642 exhibited a broader adaptive capacity by maintaining ionic stability and antioxidant protection under saline and combined stresses, while preserving the ability to activate defense-related metabolic responses under pathogen pressure.

These findings carry important implications for wheat breeding programs in saline and disease-prone regions. Breeders should consider a combination of ionic and defense-related traits rather than relying on a single indicator. However, in saline environments, the shoot K^+/Na^+ ratio emerges as the most reliable and practical selection criterion. Genotypes such as 1528 and 1642, which occupy the ideal quadrants in the biplots, can be introduced as superior parents for breeding crosses aimed at combined stress tolerance.

Another notable finding of this study was the significance of the genotype $\times$ treatment interaction for all measured traits. This clearly demonstrates that the responses of different wheat genotypes to environmental stresses are not uniform, and each genotype exhibits a unique physiological and biochemical response pattern, such that the performance of a genotype under one specific stress condition (e.g., salinity) cannot reliably predict its performance under another stress (e.g., disease) or combined stress. This observation is fully consistent with extensive evidence from other crop species.

In a study on cucumber, Chojak-Koźniewska et al. (2017) clearly showed that plant responses to combined salinity and pathogen stress are specific and distinct from the sum of responses to each stress individually. They found that salinity stress, acting as a dominant stressor, weakened plant defense mechanisms against the pathogen, making salt-treated plants more susceptible to bacterial (*Pseudomonas syringae*) infection. This pattern, where salinity acts as a dominant stress and suppresses secondary metabolic defense pathways, is precisely consistent with our observations in wheat, where under combined stress, salinity shifted the plant's priority from costly defense pathways (such as the PAL pathway) toward ionic regulation [30].

Similarly, Bai et al. (2018), in a comprehensive study on tomato under combined salinity and powdery mildew stress, concluded that the nature of plant responses to combined stress strongly depends on salinity intensity and the type of resistance genes involved. They demonstrated that salinity stress can attenuate disease resistance in some genotypes while disease resistance remains completely stable in others [31]. This concept, that "the stability of disease resistance under salinity stress depends on the genetic resistance mechanism," well explains the diversity of genotypic responses in our study. For instance, genotype 1528, which primarily relied on an ionic–antioxidant regulation strategy, performed better under salinity and combined stress, whereas genotype 9033, which exhibited excellent defense performance under disease-alone conditions by activating the PAL pathway, showed relatively weaker performance under salinity and combined stress (due to PAL pathway suppression in the presence of salinity).

Furthermore, both research groups [30, 31] emphasized the central role of hormonal cross-talk (particularly the antagonism between abscisic acid and salicylic acid) in determining the fate of plant responses to combined stresses. They showed that salinity stress, through increasing ABA, can inhibit SA signaling and reduce the expression of defense-related genes such as PR1. These findings well explain the complex changes we observed in PAL and POD activities and total protein content across the four environments.

Collectively, this evidence indicates that the effects of combined stresses are often non-additive and cannot be predicted solely from data obtained from individual stresses. Therefore, as explicitly recommended by Bai et al. (2018), achieving cultivars resistant to combined stresses requires conducting genetic and physiological studies precisely under combined stress conditions. Selecting superior genotypes solely based on their performance under control conditions or a single stress may lead to the selection of genotypes that do not perform well under real field conditions, where combinations of stresses are common [31]. Accordingly, the findings of the present study emphasize the necessity of screening genotypes under combined stress conditions and using composite indices (such as shoot K^+/Na^+ ratio, POD and PAL activities) rather than a single indicator for selecting superior genotypes. Based on the physiological, biochemical, and multivariate analyses, a conceptual model is proposed to summarize the adaptive strategies of bread wheat under salinity stress, *Gaeumannomyces tritici* infection, and their combined effects (Figure 14). The model indicates that wheat plants preferentially activate the phenylpropanoid-based defense pathway under pathogen infection alone, whereas salinity shifts the response toward ionic homeostasis and antioxidant regulation. Under combined stress, salinity appears to become the dominant physiological constraint, resulting in prioritization of ionic regulation over secondary metabolic defense. Overall, the present study demonstrates that tolerance to simultaneous salinity and *Gaeumannomyces tritici* infection is governed by coordinated regulation of ionic homeostasis, antioxidant protection, and stress-induced metabolic adjustment rather than by a single resistance mechanism. Integration of physiological traits with multivariate approaches provides a robust framework for identifying wheat genotypes with stable performance under complex field environments. Among the evaluated genotypes, 1528 and 1642 showed the most balanced adaptive responses and represent promising genetic resources for improving combined stress tolerance in wheat breeding programs.

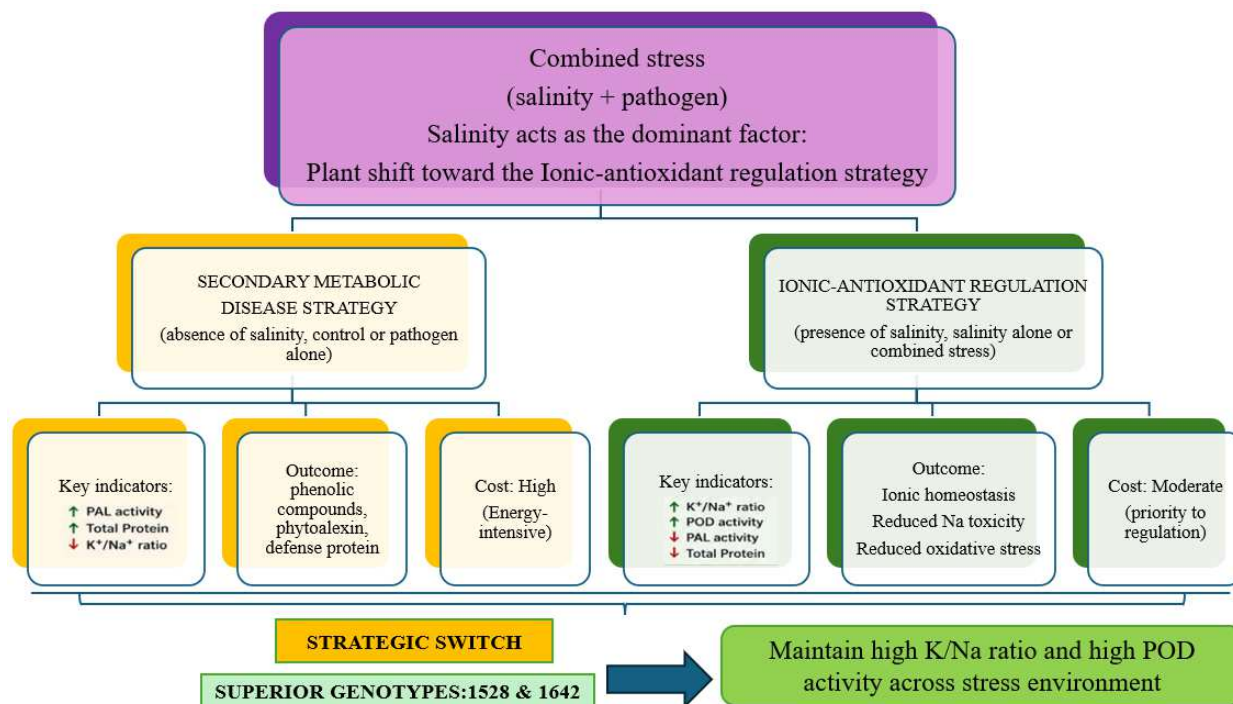


Figure 14- Conceptual model illustrating the strategic shift between secondary metabolic defense and ionic-antioxidant regulation in wheat under salinity stress, *Gaeumannomyces tritici* infection, and combined stresses. Green arrows indicate activated pathways; red dashed arrows indicate suppressed pathways. Genotypes 1528 and 1642 are highlighted as superior performers across all stress environments

5. Conclusion

The present study demonstrated that wheat responses to salinity and *Gaeumannomyces tritici* infection are highly genotype- and stress-dependent, with distinct physiological strategies emerging under different stress scenarios. Multivariate analyses revealed two major adaptive patterns: an ionic-antioxidant regulation strategy, characterized by improved K⁺/Na⁺ homeostasis and enhanced POD activity, and a secondary metabolic defense strategy, associated with increased PAL activity and total protein accumulation.

Under salinity and combined salinity-disease stress, genotypes tended to prioritize ionic regulation, suggesting that maintenance of K⁺/Na⁺ balance represents a critical component of tolerance under saline environments. In contrast, pathogen infection alone primarily stimulated metabolic defense responses involving PAL activity and protein accumulation. Under combined stress, the physiological response pattern indicated that salinity imposed a strong constraint, shifting plant priorities toward ionic homeostasis rather than activation of energetically costly secondary metabolic pathways.

Among the evaluated genotypes, 1528 and 1642 exhibited the most favorable combination of traits under combined stress, maintaining higher shoot and root K⁺/Na⁺ ratios together with effective POD activity. These genotypes also retained the capacity to activate defense-related responses under pathogen pressure, indicating a broader adaptive potential. Therefore, they represent promising genetic resources for breeding programs targeting wheat production in saline and take-all-prone environments.

From a breeding perspective, the shoot K⁺/Na⁺ ratio emerged as a reliable physiological indicator for selecting genotypes with improved performance under saline and combined stresses. However, selection based on a single trait may be insufficient; integrating ionic traits (K⁺/Na⁺ balance) with biochemical indicators such as POD and PAL activities provides a more comprehensive approach for identifying stable genotypes.

A limitation of this study is that the evaluation was conducted under controlled conditions using a limited number of physiological and biochemical parameters. Although PCA and cluster analysis provided strong evidence for distinct adaptive strategies, the underlying molecular mechanisms, including regulation of ion transporter genes, phenylpropanoid pathway genes, and stress-related signaling networks, require further investigation. Future studies

under field conditions with a larger diversity of genotypes and multi-environment validation are necessary to confirm the stability and practical value of the identified tolerance indicators.

Overall, this study provides new insights into the physiological strategies employed by wheat under simultaneous salinity and disease stress and highlights the importance of considering combined stress responses rather than individual stress tolerance when developing resilient wheat cultivars.

Acknowledgement

The authors acknowledge Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran.

Author contributions

Conceptualization: Roohallah Saberi Riseh, Hossein Dashti, and Mozhgan Gholizadeh Vazvani. Data curation: Mozhgan Gholizadeh Vazvani. Formal analysis: Mozhgan Gholizadeh Vazvani. Investigation: Mozhgan Gholizadeh Vazvani. Methodology: Mozhgan Gholizadeh Vazvani. Resources: Mozhgan Gholizadeh Vazvani. Software: Mozhgan Gholizadeh Vazvani. Supervision: Roohallah Saberi Riseh and Hossein Dashti. Validation: Hossein Dashti, Roohallah Saberi Riseh, and Evelin Loit-Harro. Visualization: Mozhgan Gholizadeh Vazvani. Writing - original draft: Mozhgan Gholizadeh Vazvani. Writing - review & editing: Roohallah Saberi Riseh, Hossein Dashti, Mozhgan Gholizadeh Vazvani, and Evelin Loit-Harro.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data Availability

the datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics and Consent to Participate declarations: not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Consent for publication

Not applicable.

References

1. Molotoks A, Smith P, Dawson TP: **Impacts of land use, population, and climate change on global food security**. *Food and energy security* 2021, **10**(1):e261.
2. Iriti M, Vitalini S: **Sustainable crop protection, global climate change, food security and safety—plant immunity at the crossroads**. *Vaccines* 2020, **8**(1):42.
3. Chakraborty S, Newton AC: **Climate change, plant diseases and food security: an overview**. *Plant pathology* 2011, **60**(1):2-14.

4. Arora G, Feng H, Anderson CJ, Hennessy DA: **Evidence of climate change impacts on crop comparative advantage and land use.** *Agricultural Economics* 2020, **51**(2):221-236.
5. Munns R, Tester M: **Mechanisms of salinity tolerance.** *Annu Rev Plant Biol* 2008, **59**:651-681.
6. Freeman J, Ward E: **Gaeumannomyces graminis, the take-all fungus and its relatives.** *Molecular Plant Pathology* 2004, **5**(4):235-252.
7. Mcmillan V, Hammond-Kosack K, Gutteridge R: **Evidence that wheat cultivars differ in their ability to build up inoculum of the take-all fungus, Gaeumannomyces graminis var. tritici, under a first wheat crop.** *Plant Pathology* 2010, **60**(2):200-206.
8. Palma-Guerrero J, Chancellor T, Spong J, Canning G, Hammond J, Mcmillan V, Hammond-Kosack K: **Take-all disease: new insights into an important wheat root pathogen.** *Trends in Plant Science* 2021, **26**(8):836-848.
9. Cuin TA, Betts SA, Chalmandrier R, Shabala S: **A root's ability to retain K⁺ correlates with salt tolerance in wheat.** *Journal of experimental botany* 2008, **59**(10):2697-2706.
10. Munns R, James RA, Läuchli A: **Approaches to increasing the salt tolerance of wheat and other cereals.** *Journal of experimental botany* 2006, **57**(5):1025-1043.
11. El-Hendawy SE, Hassan WM, Al-Suhaibani NA, Refay Y, Abdella KA: **Comparative performance of multivariable agro-physiological parameters for detecting salt tolerance of wheat cultivars under simulated saline field growing conditions.** *Frontiers in plant science* 2017, **8**:435.
12. Das SK, Patra JK, Thatoi H: **Antioxidative response to abiotic and biotic stresses in mangrove plants: A review.** *International Review of Hydrobiology* 2016, **101**(1-2):3-19.
13. Mittler R: **Oxidative stress, antioxidants and stress tolerance.** *Trends in plant science* 2002, **7**(9):405-410.
14. Srivastava M, Shahi S: **Effect of salinity on morpho-physiological aspects, antioxidant enzymatic studies and yield attributes in wheat genotypes.** *Indian Journal of Plant Physiology* 2018, **23**(2):385-392.
15. Bindschedler LV, Dewdney J, Blee KA, Stone JM, Asai T, Plotnikov J, Denoux C, Hayes T, Gerrish C, Davies DR: **Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance.** *The Plant Journal* 2006, **47**(6):851-863.
16. Al-Khayri JM, Rashmi R, Toppo V, Chole PB, Banadka A, Sudheer WN, Nagella P, Shehata WF, Al-Mssallem MQ, Alessa FM: **Plant secondary metabolites: the weapons for biotic stress management.** *Metabolites* 2023, **13**(6):716.
17. Li L, Hao R, Yang X, Feng Y, Bi Z: **Piriformospora indica increases resistance to Fusarium pseudograminearum in wheat by inducing phenylpropanoid pathway.** *International Journal of Molecular Sciences* 2023, **24**(10):8797.
18. Astanek RK, Bolandnazar S, Nahandi FZ, Oustan S: **Effect of selenium application on phenylalanine ammonia-lyase (PAL) activity, phenol leakage and total phenolic content in garlic (Allium sativum L.) under NaCl stress.** *Information processing in Agriculture* 2018, **5**(3):339-344.
19. Wu X, Zhu S, He L, Cheng G, Li T, Meng W, Wen F: **Phenylalanine Ammonia-Lyase: A Core Regulator of Plant Carbon Metabolic Flux Redistribution—From Molecular Mechanisms and Growth Modulation to Stress Adaptability.** *Plants* 2025, **14**(24):3811.
20. Neilson EH, Goodger JQ, Woodrow IE, Møller BL: **Plant chemical defense: at what cost?** *Trends in plant science* 2013, **18**(5):250-258.
21. Dunn DC, Duncan LW, Romeo JT: **Changes in arginine, pal activity, and nematode behavior in salinity-stressed citrus in honour of Professor GH Neil Towers 75th birthday.** *Phytochemistry* 1998, **49**(2):413-417.
22. Mittler R: **Abiotic stress, the field environment and stress combination.** *Trends in plant science* 2006, **11**(1):15-19.

23. Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R: **Abiotic and biotic stress combinations.** *New Phytologist* 2014, **203**(1):32-43.
24. Kissoudis C, van de Wiel C, Visser RG, van der Linden G: **Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk.** *Frontiers in plant science* 2014, **5**:207.
25. Souman M, Kostandi S: **Effect of saline environment on yield and smut disease severity of different corn genotypes (Zea mays L.).** *Journal of Phytopathology* 1998, **146**(4):185-189.
26. Kissoudis C, Sunarti S, Van De Wiel C, Visser RG, van der Linden CG, Bai Y: **Responses to combined abiotic and biotic stress in tomato are governed by stress intensity and resistance mechanism.** *Journal of experimental botany* 2016, **67**(17):5119-5132.
27. Wiese J, Kranz T, Schubert S: **Induction of pathogen resistance in barley by abiotic stress.** *Plant Biology* 2004, **6**(05):529-536.
28. Pastori GM, Foyer CH: **Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls.** *Plant physiology* 2002, **129**(2):460-468.
29. Shah AH, Ahmad S, Durrani H, Shah S: **Cross adaptation; An alternate approach of genetic engineering for salinity tolerance in rice cell lines.** *Plant Cell Biotechnol Mol Biol* 2017, **18**:39-48.
30. Chojak-Koźniewska J, Linkiewicz A, Sowa S, Radzioch M, Kuźniak E: **Interactive effects of salt stress and Pseudomonas syringae pv. lachrymans infection in cucumber: involvement of antioxidant enzymes, abscisic acid and salicylic acid.** *Environmental and experimental botany* 2017, **136**:9-20.
31. Bai Y, Kissoudis C, Yan Z, Visser RG, Van der Linden G: **Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress.** *The plant journal* 2018, **93**(4):781-793.
32. Bostock RM, Pye MF, Roubtsova TV: **Predisposition in plant disease: exploiting the nexus in abiotic and biotic stress perception and response.** *Annual review of phytopathology* 2014, **52**(1):517-549.
33. Zhang H, Sonnewald U: **Differences and commonalities of plant responses to single and combined stresses.** *The Plant Journal* 2017, **90**(5):839-855.
34. Gholizadeh Vazvani M, Dashti H, Saberi Riseh R, Loit E: **Association analysis of response to take-all disease with agronomic traits and molecular markers and selection ideal genotypes in bread wheat (Triticum aestivum L.) genotypes.** *Molecular Breeding* 2025, **45**(4):36.
35. Gholizadeh Vazvani M, Saberi Riseh R, Dashti H, Jaffaraghaei M, Loit-Harro E: **Integration of HMW-GS and retrotransposon marker LTR14 for selection of wheat genotypes combining baking quality and resistance to take-all disease.** *BMC plant biology* 2026.
36. Hussain N, Sohail Y, Shakeel N, Javed M, Bano H, Gul HS, Zafar ZU, Frahat Zaky Hassan I, Ghaffar A, Athar H-u-R: **Role of mineral nutrients, antioxidants, osmotic adjustment and PSII stability in salt tolerance of contrasting wheat genotypes.** *Scientific Reports* 2022, **12**(1):12677.
37. Bradford MM: **A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.** *Analytical biochemistry* 1976, **72**(1-2):248-254.
38. D'Cunha GB, Satyanarayan V, Nair PM: **Stabilization of phenylalanine ammonia lyase containing Rhodotorula glutinis cells for the continuous synthesis of L-phenylalanine methyl ester/96.** *Enzyme and Microbial Technology* 1996, **19**(6):421-427.
39. Plewa MJ, Smith SR, Wagner ED: **Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase.** *Mutation research/fundamental and molecular mechanisms of mutagenesis* 1991, **247**(1):57-64.

40. Ibrahim AA, Fakhr MA, Ramadan S, Basuoni MM, Abdel-Haleem M: **Transcriptional signatures of salinity tolerance in Egyptian wheat: unveiling WRKY-mediated defense mechanisms.** *BMC Plant Biology* 2026.
41. Bhutta W: **Antioxidant activity of enzymatic system of two different wheat (*Triticum aestivum* L.) cultivars growing under salt stress.** 2011.
42. Omar AAA, Zaman MMU, Abedin MT, Alam MS, Khatun MA, Akter N, Sayed MA: **Unraveling the dynamics of salt stress response in wheat cultivars: Insights into growth inhibition, antioxidant enzyme activity, and AOX gene expression.** *Journal of Crop Science and Biotechnology* 2024, **27**(4):439-448.
43. Singh M, Pandita S, Sushmita, Upadhyay SK, Verma PC: **Genomic and Functional Characterization of the Phenylalanine Ammonia Lyase (PAL) Gene Family in Wheat (*Triticum aestivum*) Reveals Roles in Salt Stress Adaptation.** *Biotechnology and Applied Biochemistry* 2025.
44. Cowan M: **Water use and phosphorus and potassium status of wheat seedlings colonized by *Gaeumannomyces graminis* or *Phialophora radiculicola*.** *Plant and Soil* 1979, **52**(1):1-8.
45. Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman SD, Tester M: **Wheat grain yield on saline soils is improved by an ancestral Na⁺ transporter gene.** *Nature biotechnology* 2012, **30**(4):360-364.
46. Shahzad B, Yun P, Shabala L, Zhou M, Sellamuthu G, Venkataraman G, Chen Z-H, Shabala S: **Unravelling the physiological basis of salinity stress tolerance in cultivated and wild rice species.** *Functional Plant Biology* 2022, **49**(4):351-364.
47. Sairam RK, Rao KV, Srivastava G: **Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration.** *Plant science* 2002, **163**(5):1037-1046.
48. Dixon RA, Achnine L, Kota P, Liu CJ, Reddy MS, Wang L: **The phenylpropanoid pathway and plant defence—a genomics perspective.** *Molecular plant pathology* 2002, **3**(5):371-390.
49. Alshaharni MO: **Molecular, biochemical and physiological responses of wheat (*Triticum aestivum*) to spot blotch disease and salinity.** Newcastle University; 2022.
50. Liu A, Xiao Z, Wang Z, Lam H-M, Chye M-L: **Galactolipid and phospholipid profile and proteome alterations in soybean leaves at the onset of salt stress.** *Frontiers in Plant Science* 2021, **12**:644408.
51. Zhang S, Wen X, Zhou G, Cui P, Li F, Han Y, Wang Y: **Physiological differences between Mo17 and B73 maize inbred lines under salt stress.** *New Zealand Journal of Botany* 2025, **63**(5):1456-1467.