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Unravelling wheat genotypic responses: insights into salinity stress tolerance in relation to oxidative stress, antioxidant mechanisms, osmolyte accumulation and grain quality parameters

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Abstract

Background Salt stress is a prominent abiotic stressor that imposes constraints on grain yield and quality across various crops, including wheat (*Triticum aestivum*). This study focused on assessing the genetic diversity of 20 wheat genotypes categorized as tolerant, moderately tolerant, and sensitive with three genotypes of unknown tolerance. To address salinity stress-related problems, different morpho-physiological, osmoprotectant, biochemical, yield, and grain quality-related parameters were analyzed under control (pH 8.0, EC 3.9) and saline-sodic (pH 9.4, EC 4.02) conditions in field.

Results Findings revealed noteworthy variations among the genotypes in response to salinity stress. Greater accumulation of Na^+ and lower K^+ content were observed in response to salt stress in the sensitive varieties HD1941 and K9162. Proline, a stress indicator, exhibited significantly ($p \leq 0.05$) greater accumulation in response to salinity stress, particularly in the tolerant cultivars KRL210 and KH65. Salt stress induced the most significant decrease ($p \leq 0.05$) in spike length, thousand-grain weight, and hectolitre weight coupled with increased protein content in sensitive varieties, resulting in diminished yield.

Conclusion Correlation analysis of parameters under salinity stress showed that SOD, proline, and K^+ contents can be used as the most efficient screening criteria for salinity stress during early developmental stages. Principal component analysis revealed that DBW187, DBW303, and DBW222 varieties were tolerant to salinity stress and exhibited an effective antioxidant system against salinity. This study will facilitate salt-tolerant wheat breeding in terms of the identification of tolerant lines by screening for limited traits in a wide range of germplasms.

Keywords Salt tolerance, Sodium ions, Potassium ions, Proline, Oxidative stress, Sedimentation value

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Background

Wheat (*Triticum aestivum* L.) is a major staple food crop that contributes significantly to food and nutritional security worldwide. Wheat provides approximately 30% of the world's population with 23.8% calories and 33.06% protein requirements [1]. Abiotic stresses such as salt, heat, and drought create a suboptimal environment and have a negative impact on the yield and productivity of wheat [2]. Salinity stress in arid and semi-regions of the world leads to a loss in agricultural productivity because of the accumulation of salts in the soil [3]. At any stage of crop growth, salt stress causes irreversible losses in yield potential in various crops, including wheat, barley, and rice [4]. Almost 7 million hectares of Indian soil are now contaminated by salt, and by 2050, salinization of the soil will result in the loss of 50% of cultivable land globally [5].

In salt-affected soils, excess Na^+ ions are present at exchange sites, and high concentrations of carbonate and bicarbonate anions are associated with high pH. High concentrations of Na^+ ions in soils compete for K^+ uptake, which leads to K^+ deficiency and promotes K^+ leakage from cells. This K^+-induced deficiency inhibits the growth of plants because it plays an important role in maintaining cell turgor, membrane potential, and enzymatic activities [6]. Salinity stress is responsible for the generation of osmotic and ionic stresses, the influx of large amounts of Na^+ inside plant cells, and increased Na^+ concentrations in the cytoplasm and vacuole, which are toxic to metabolic mechanisms and lead to cell death.

Wheat production is severely hampered by soil salinity, which reduces growth and productivity by lowering water uptake and creating nutritional deficiencies through ion toxicity [7]. Salinity stress leads to a significant reduction in photosynthetic performance and weakens the photosynthetic pigments present in the thylakoid membrane of the chloroplast. High salt toxicity in soil impairs the generation of photosynthetic pigments because of the inhibitory effect of salt ions on the biosynthesis of various chlorophyll components [8]. The chlorophyll content (CC), relative water content (RWC), normal difference vegetation index (NDVI), and membrane stability index (MSI) in wheat were negatively impacted by soil salinity. Wheat plants are less able to absorb nutrients from roots as the salinity increases because less water is accessible to roots.

In response to salinity stress, primary plant activities, including protein synthesis, energy production and lipid metabolism, change during the plant's embryonic phases [9, 10]. As a result of the plant's significant growth retardation, toxic metabolite synthesis and molecular damage can cause plant death [11]. Plants have developed internal resistance mechanisms to scavenge the harmful effects of reactive oxygen species (ROS), such as superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen (O_2), generated due to salinity stress [12]. To combat stress, plants modify their metabolism and enzymatic and non-enzymatic antioxidant mechanisms. Plants have also evolved various biochemical defences against osmotic and ionic stress, including osmotic tolerance, ion exclusion, tissue tolerance, synthesis of compatible solutes, amino acids, and organic acids, adjustments to membrane structure, induction of antioxidant defence, and hormone homeostasis [4, 8]. Reactive oxygen species scavenging enzymes, which are part of the antioxidant defence, play a direct role in the degradation of ROS, including CAT (catalase), SOD (superoxide dismutase), GR (glutathione reductase), and APX (ascorbate peroxidase) [13]. SOD dismutases O_2^- into H_2O_2, which is further catalyzed into H_2O and O_2 by CAT and APX [14]. Furthermore, to overcome the osmotic stress induced by salinity, plants synthesize and accumulate metabolically compatible solutes such as proline, sugars, and betaines. Proline acts as an osmoprotectant and helps plants maintain tissue water potential lower than that of the soil to maintain turgor pressure for growth [7]. In addition to osmotic balance, these compatible solutes also act as antioxidants and help to stabilize subcellular structures, buffering the cellular redox potential against stress [15].

Wheat grain yield is a quantitative trait affected by salinity stress. Salinity stress suppresses the growth and development of spikes at the reproductive and maturity stages. Plant height, spike length, and grain weight are negatively affected by salinity stress. The ability of wheat to produce unique baked products, such as bread and chapatti, depends on grain quality. This attribute is assessed by physical and compositional properties such as grain hardness (GH), protein content (PC), and moisture content (MC) [16]. Affect of salt stress on wheat quality attributes is a relatively unexplored avenue, thus a two years study was proposed to assess the effects of salinity stress on physiological, biochemical, yield, and other quality-related parameters in 20 wheat genotypes with different genetic backgrounds to identify parameters that can be used as biomarkers for rapid selection and improvement of salt-tolerant cultivars. In this study we hypothesized that the response of wheat quality to salt stress would differ among wheat genotypes. Correlation analysis and PCA analysis was carried out to understand the variations in adaptive mechanisms in wheat plants under control and salt stress conditions. The lack of precise indices of physiological and agronomic traits related to salinity stress and the low genetic variability in the currently available wheat germplasm lines are among the main reasons for the limited success in breeding salt-tolerant varieties. Previously reported salt-tolerant varieties have been used for the breeding of crops, but because of a lack of understanding of the mechanism of salt tolerance, less success has been achieved. Most saline-prone

areas remain largely uncultivated. Growing salt-tolerant wheat on saline soils could be a significant attempt to use these areas and to meet the demands of the ever-increasing population to satisfy their food needs. This would be possible by understanding the mechanism behind the variations in the tolerance of different wheat cultivars and by identifying salt-tolerant genotypes.

Results

Agro-morphology and physiological responses of wheat to saline-sodic conditions

The agronomic and physiological responses of the wheat genotypes significantly differed under salinity stress. In this study, a significant reduction in agronomic traits during the early flag leaf stage and maturity stage of plants was observed. Significant growth and biomass reductions were observed in twenty wheat cultivars grown under salt stress. All physiological traits, such as plant height, chlorophyll content, SPAD, NDVI, RWC, and MSI, showed significant ($p \leq 0.05$) reductions except in the tolerant cultivars under salinity stress compared to their respective controls. The overall means and ranges of these traits for the 20 genotypes under both control and stress conditions are shown in Supplementary Table 2. Salt stress significantly affects the plant height of wheat genotypes. The highest plant height was found in DBW187 (100.00 cm), and the least plant height was found in GW89 (66.00 cm) under salt stress. The highest percent reduction in plant height was observed for the sensitive varieties HD2009 (-32.03%) and GW89 (-29.29%), followed by the moderate varieties (Table 1), whereas the highest percent increase in plant height was observed for DBW187 (4.20%), the tolerant varieties KRL210 (1.19%), and AJANTA (1.50%) compared to the control under salinity stress (Supplementary Table 2, Fig. 1). The maximum SPAD chlorophyll value was observed in AJANTA (46.60), and the minimum was observed in the sensitive variety HD2009 (38.10) under salt stress. The SPAD chlorophyll value decreased the most in the sensitive variety K9162 (-12.55%) and HD2009 (-12.01%), and a significant percentage increase was observed in the tolerant variety KRL210 (2.69%) and AJANTA (1.75%) compared to the control under salt stress (Supplementary Table 2).

The water status of the plant is expressed by the RWC. The highest relative water content was detected in DBW 17 (85.04%), and the lowest relative water content (41.90%) was detected in sensitive HD1941 under salt stress. In our study, the RWC decreased significantly in some moderate and sensitive wheat cultivars under stress, but greater reductions were observed in sensitive cultivars (Fig. 1). The greatest percent reduction was observed in the sensitive varieties HD1941 (-85.48%) and K9162 (-76.93%), whereas an increase in percent change was observed for DBW222 (7.46%), KH65 (5.42%), and DBW17 (0.21%) compared to the control under salt stress (Supplementary Table 2). The NDVI was measured, and a maximum value was observed in DBW222 (0.71) and a minimum in HD1941 (0.21) under salt stress (Fig. 1). The greatest percent reduction was measured in HD1941 (-59.75%) and K9162 (-44.86%), and the lowest was observed in KRL 210 (-0.55%) and DBW187 (-1.43%) compared to the control (Table 1). The maximum MSI was observed in DBW222 (88.64%), and the minimum was found in GW89 (44.01) (Fig. 1). A greater percentage of the reduction in MSI was observed in the sensitive varieties GW89 (-42.90%), HD3086 (-27.84%), and K9162 (-26.50%) than in the control. The tolerant varieties KRL210 (5.74%) and PBW502 (5.67%) can maintain the integrity of the plasma membrane more efficiently (Supplementary Table 2). The maximum total chlorophyll accumulation was found in KRL210 (11.62 mg/gFW), and the minimum was found in GW89 (3.97 mg/gFW) under salt stress. A decrease in the total chlorophyll content percentage was detected in the sensitive varieties K9162 (-39.29%) and GW89 (-38.13%), whereas an increase was detected in the tolerant varieties AJANTA (3.07%) and KRL210 (0.18%), and a smaller reduction was detected in the moderate variety PBW65 (-19.96%) under salt stress compared with the control (Supplementary Table 3, Fig. 2).

Biochemical parameters

The oxidative stress markers H_2O_2 and MDA increased significantly ($p < 0.05$) under salinity stress. Salt stress led to the accumulation of H_2O_2 in all the wheat genotypes. The sensitive genotype K9162 showed the highest accumulation of H_2O_2 (7.56 µmol/g FW), and the lowest was found in KRL210 (4.82 µmol/g FW) under salt stress (Supplementary Table 4). The greatest percent increase was observed for the sensitive varieties K9162 (94.96%) and HD2009 (62.02%), and the least percent increase was observed for the tolerant and of unknown tolerance levels and for the moderate varieties KRL210 (13.75%), DBW222 (15.97%), and KH65 (19.02%) (Supplementary Table 4, Fig. 3). MDA is used as a biomarker of the response to oxidative stress. Salt stress significantly increased the MDA content in all the wheat genotypes. The highest MDA accumulation was observed in the sensitive variety HD2009 (8.38 µM/g FW), and the lowest was observed in the tolerant variety KRL210 (3.99 µM/g FW) under salt stress. The greatest percent increase was observed among the sensitive varieties K9162 (71.54%) and GW89 (70.92%), and the greatest percent reduction was observed among the tolerant varieties KRL210 (-24.35%) and KH65 (-11.70%) compared to the control under salt stress conditions (Supplementary Table 4, Fig. 3).

Table 1 Changes in yield parameters of 20 wheat genotypes exposed to salinity stress conditions. The values are expressed as the means ± SDs ($n = 3$). The figures in the brackets depict the relative percentage of change from the control

	Length of spike (cm)		Grain Weight/spike		TGW (gm)		Protein (%)		Moisture (%)		Hectoliter weight (gm)	
Genotypes	Normal	Salt	Normal	Salt	Normal	Salt	Normal	Salt	Normal	Salt	Normal	Salt
AJANTA	11.17 ± 0.29 ^{ab}	12.17 ± 0.29 (8.96) ^{abcd}	1.96 ± 0.06 ^{e}	2.11 ± 0.04 (7.38) ^{c}	30.01 ± 2.08 ^{defg}	31.46 ± 0.73 ^{ef} (4.85)	14.46 ± 0.42 ^{cde}	14.69 ± 0.23 ^{de} (1.59)	10.68 ± 0.56 ^{ab}	10.48 ± 0.59 ^{abcd} (-1.87)	73.25 ± 0.49 ^{cd}	75.90 ± 2.54 ^{ab} (3.62)
KH65	11.73 ± 0.64 ^{ab}	11.36 ± 0.31 (-3.18) ^{cdefg}	2.06 ± 0.12 ^{d}	2.06 ± 0.07 (-0.24) ^{cd}	37.49 ± 0.42 ^{a}	39.21 ± 1.17 ^{a} (4.60)	15.26 ± 0.24 ^{bc}	16.04 ± 0.23 ^{b} (5.11)	10.53 ± 0.49 ^{ab}	9.93 ± 0.62 ^{cdef} (-5.65)	78.60 ± 0.42 ^{a}	76.10 ± 0.99 ^{a} (-3.18)
KRL210	11.67 ± 0.76 ^{ab}	13.77 ± 0.64 (18.00) ^{a}	2.26 ± 0.11 ^{c}	2.66 ± 0.06 (17.92) ^{b}	33.19 ± 0.67 ^{bc}	37.02 ± 0.93 ^{ab} (11.57)	14.86 ± 0.38 ^{cd}	15.2 ± 0.10 ^{def} (2.29)	11.34 ± 0.14 ^{a}	10.69 ± 0.31 ^{ab} (-1.38)	69.25 ± 0.63 ^{hij}	71.90 ± 0.57 ^{cde} (3.82)
DWR162	11.77 ± 0.75 ^{ab}	13.67 ± 0.76 (16.15) ^{a}	1.66 ± 0.07 ^{g}	1.99 ± 0.10 (19.88) ^{d}	31.82 ± 1.29 ^{cde}	31.16 ± 0.63 ^{efg} (-2.06)	14.31 ± 0.05 ^{cdef}	14.77 ± 0.27 ^{de} (3.18)	11.32 ± 0.28 ^{a}	11.10 ± 0.12 ^{a} (-1.99)	67.60 ± 0.85 ^{j}	65.60 ± 1.13 ^{hij} (-2.96)
GW190	11.50 ± 0.87 ^{ab}	10.33 ± 0.29 (-10.14) ^{efgh}	1.22 ± 0.06 ^{k}	1.27 ± 0.07 (4.10) ^{gh}	27.10 ± 1.66 ^{hij}	28.92 ± 0.72 ^{ghi} (6.72)	15.24 ± 0.30 ^{bc}	15.67 ± 0.40 ^{cd} (2.82)	10.54 ± 0.46 ^{ab}	9.83 ± 0.06 ^{def} (-6.69)	69.83 ± 0.81 ^{ghi}	68.35 ± 0.49 ^{fgh} (-2.12)
GW503	10.50 ± 0.50 ^{b}	12.67 ± 0.76 (20.63) ^{abc}	0.89 ± 0.11 ^{m}	1.00 ± 0.12 (12.92) ^{j}	28.39 ± 0.69 ^{fghij}	29.23 ± 1.05 (2.99) ^{fghi}	14.94 ± 0.72 ^{cd}	15.12 ± 0.14 ^{def} (1.17)	10.84 ± 0.97 ^{ab}	10.58 ± 0.78 ^{abc} (-2.40)	74.10 ± 0.42 ^{bc}	72.15 ± 0.49 ^{cd} (-2.63)
DBW17	10.50 ± 0.50 ^{b}	12.50 ± 0.50 (19.05) ^{abc}	1.63 ± 0.06 ^{gh}	1.49 ± 0.09 (-8.90) ^{f}	32.05 ± 1.55 ^{cde}	30.20 ± 1.18 ^{efgh} (-5.77)	14.89 ± 0.05 ^{cd}	15.24 ± 0.51 ^{def} (2.32)	10.78 ± 0.78 ^{ab}	10.47 ± 0.42 ^{abcd} (-2.83)	72.75 ± 0.35 ^{cde}	72.03 ± 0.38 ^{cd} (-0.99)
NI5643	11.93 ± 51 ^{ab}	13.57 ± 0.60 (13.69) ^{ab}	1.59 ± 0.07 ^{hi}	1.20 ± 0.04 (-24.53) ^{hi}	26.68 ± 1.19 ^{ij}	25.39 ± 0.54 ^{jk} (2.84)	16.28 ± 0.71 ^{a}	16.64 ± 0.28 ^{a} (2.18)	11.19 ± 0.28 ^{a}	10.57 ± 0.37 ^{abc} (-5.59)	69.00 ± 1.13 ^{ij}	68.90 ± 1.27 ^{efg} (-0.14)
NW1014	10.33 ± 0.29 ^{b}	11.33 ± 0.58 (9.68) ^{cdef}	1.92 ± 0.04 ^{e}	1.48 ± 0.06 (-23.18) ^{f}	26.38 ± 2.65 ^{ij}	22.80 ± 0.98 ^{lm} (-13.55)	14.91 ± 0.18 ^{cd}	15.76 ± 0.17 ^{c} (5.70)	10.81 ± 0.09 ^{ab}	10.28 ± 0.13 ^{bcd} (-4.99)	69.30 ± 0.42 ^{hi}	62.70 ± 0.42 ^{jk} (-9.52)
PBW65	11.17 ± 0.29 ^{ab}	11.67 ± 0.29 (4.48) ^{cdef}	2.58 ± 0.08 ^{a}	1.81 ± 0.05 (-29.65) ^{e}	29.72 ± 1.78 ^{efgh}	30.00 ± 0.72 ^{efghi} (0.96)	12.92 ± 0.71 ^{g}	13.35 ± 0.40 ^{g} (3.29)	10.63 ± 0.49 ^{ab}	10.21 ± 0.09 ^{bcd} (-4.00)	68.90 ± 0.14 ^{ij}	70.60 ± 0.85 ^{def} (2.47)
PBW502	10.00 ± 0.50 ^{b}	9.67 ± 0.76 (-3.33) ^{gh}	1.53 ± 0.08 ^{j}	1.78 ± 0.08 (16.29) ^{e}	30.96 ± 0.54 ^{cdef}	32.18 ± 0.84 ^{de} (3.92)	14.80 ± 0.95 ^{cd}	15.19 ± 0.15 ^{def} (2.57)	10.87 ± 0.06 ^{ab}	10.18 ± 0.32 ^{bcde} (-6.35)	75.55 ± 0.35 ^{b}	76.00 ± 1.13 ^{ab} (0.60)
DBW187	10.67 ± 0.76 ^{b}	12.40 ± 0.46 (16.25) ^{abc}	2.33 ± 0.08 ^{b}	3.10 ± 0.08 (33.26) ^{a}	32.59 ± 1.46 ^{cd}	34.02 ± 0.27 ^{cd} (4.40)	14.61 ± 0.37 ^{cde}	14.80 ± 0.12 ^{cde} (1.30)	11.05 ± 0.45 ^{a}	10.70 ± 0.47 ^{ab} (-3.26)	72.00 ± 0.71 ^{def}	74.16 ± 1.36 ^{abc} (3.00)
DBW222	11.00 ± 0.50 ^{ab}	12.73 ± 0.21 (15.76) ^{abc}	1.57 ± 0.06 ^{ij}	1.70 ± 0.06 (7.94) ^{e}	32.09 ± 1.61 ^{cde}	35.16 ± 0.29 ^{bc} (9.57)	13.42 ± 0.65 ^{fg}	14.09 ± 0.15 ^{f} (4.96)	10.66 ± 0.33 ^{ab}	10.15 ± 0.32 ^{bcde} (-4.83)	71.50 ± 0.99 ^{efg}	72.94 ± 0.91 ^{bcd} (2.02)
DBW303	11.93 ± 0.12 ^{ab}	13.07 ± 0.25 (9.50) ^{abc}	2.05 ± 0.08 ^{d}	2.10 ± 0.11 (2.20) ^{cd}	31.95 ± 1.16 ^{cde}	32.05 ± 0.81 ^{de} (0.31)	13.66 ± 0.71 ^{efg}	14.04 ± 0.25 ^{f} (2.74)	11.14 ± 0.05 ^{a}	11.05 ± 0.23 ^{a} (-0.90)	75.00 ± 0.42 ^{b}	73.53 ± 1.09 ^{abcd} (-1.96)
NW1076	11.83 ± 1.04 ^{ab}	10.50 ± 0.50 (-11.27) ^{defgh}	1.02 ± 0.06 ^{l}	1.20 ± 0.05 (16.59) ^{hi}	29.62 ± 1.21 ^{efgh}	25.07 ± 0.84 ^{kl} (-15.39)	14.49 ± 0.26 ^{cde}	15.63 ± 0.27 ^{cd} (7.83)	10.61 ± 0.35 ^{ab}	10.42 ± 0.43 ^{abcd} (-1.84)	71.70 ± 0.99 ^{def}	65.85 ± 0.21 ^{ghi} (-8.16)
HD1941	10.83 ± 0.76 ^{ab}	10.10 ± 0.85 (-6.77) ^{efgh}	0.90 ± 0.04 ^{m}	0.66 ± 0.08 (-26.67) ^{k}	28.79 ± 0.75 ^{fghi}	21.70 ± 0.68 ^{m} (-24.64)	15.29 ± 0.47 ^{abc}	16.62 ± 0.28 ^{a} (8.66)	10.54 ± 0.59 ^{ab}	9.47 ± 0.35 ^{ef} (-10.19)	70.90 ± 1.56 ^{fgh}	59.74 ± 0.76 ^{kl} (-15.74)
HD2009	11.00 ± 1.00 ^{ab}	9.43 ± 0.31 (-14.24) ^{h}	1.04 ± 0.07 ^{l}	0.71 ± 0.06 (-31.25) ^{k}	35.45 ± 0.39 ^{ab}	27.48 ± 0.57 ^{ijk} (-22.50)	14.22 ± 0.28 ^{cdef}	15.91 ± 0.14 ^{bc} (11.85)	10.04 ± 0.70 ^{b}	9.43 ± 0.15 ^{f} (-6.17)	74.15 ± 1.34 ^{bc}	65.21 ± 1.29 ^{ij} (-12.06)
HD3086	12.83 ± 0.76 ^{a}	11.83 ± 0.76 (-7.79) ^{bcde}	1.64 ± 0.10 ^{gh}	1.28 ± 0.05 (-21.65) ^{gh}	32.42 ± 1.31 ^{cd}	27.82 ± 0.67 ^{hij} (-14.19)	14.03 ± 0.21 ^{def}	14.73 ± 0.52 ^{cde} (4.95)	11.23 ± 0.18 ^{a}	10.47 ± 0.14 ^{abcd} (-6.81)	73.25 ± 1.20 ^{cd}	66.25 ± 3.04 ^{ghi} (-9.56)
GW89	11.50 ± 0.87 ^{ab}	10.00 ± 0.30 (-13.04) ^{fgh}	1.83 ± 0.05 ^{f}	1.09 ± 0.07 (-40.60) ^{ij}	26.05 ± 0.10 ^{j}	21.38 ± 0.96 ^{m} (-17.93)	14.89 ± 0.36 ^{cd}	16.67 ± 0.23 ^{a} (11.92)	11.21 ± 0.08 ^{a}	9.99 ± 0.94 ^{bcdef} (-10.97)	72.50 ± 0.42 ^{cdef}	59.90 ± 1.56 ^{kl} (-17.37)
K9162	11.67 ± 0.58 ^{ab}	9.83 ± 0.29 (-15.71) ^{gh}	1.81 ± 0.04 ^{f}	1.32 ± 0.05 (-26.80) ^{g}	28.05 ± 0.75 ^{ghij}	21.72 ± 0.65 m (-22.55)	14.52 ± 0.49 ^{cde}	16.29 ± 0.54 ^{ab} (12.15)	10.72 ± 0.52 ^{ab}	9.31 ± 0.25 ^{f} (-13.19)	68.25 ± 0.49 ^{ij}	58.05 ± 0.35 ^{l} (-14.95)

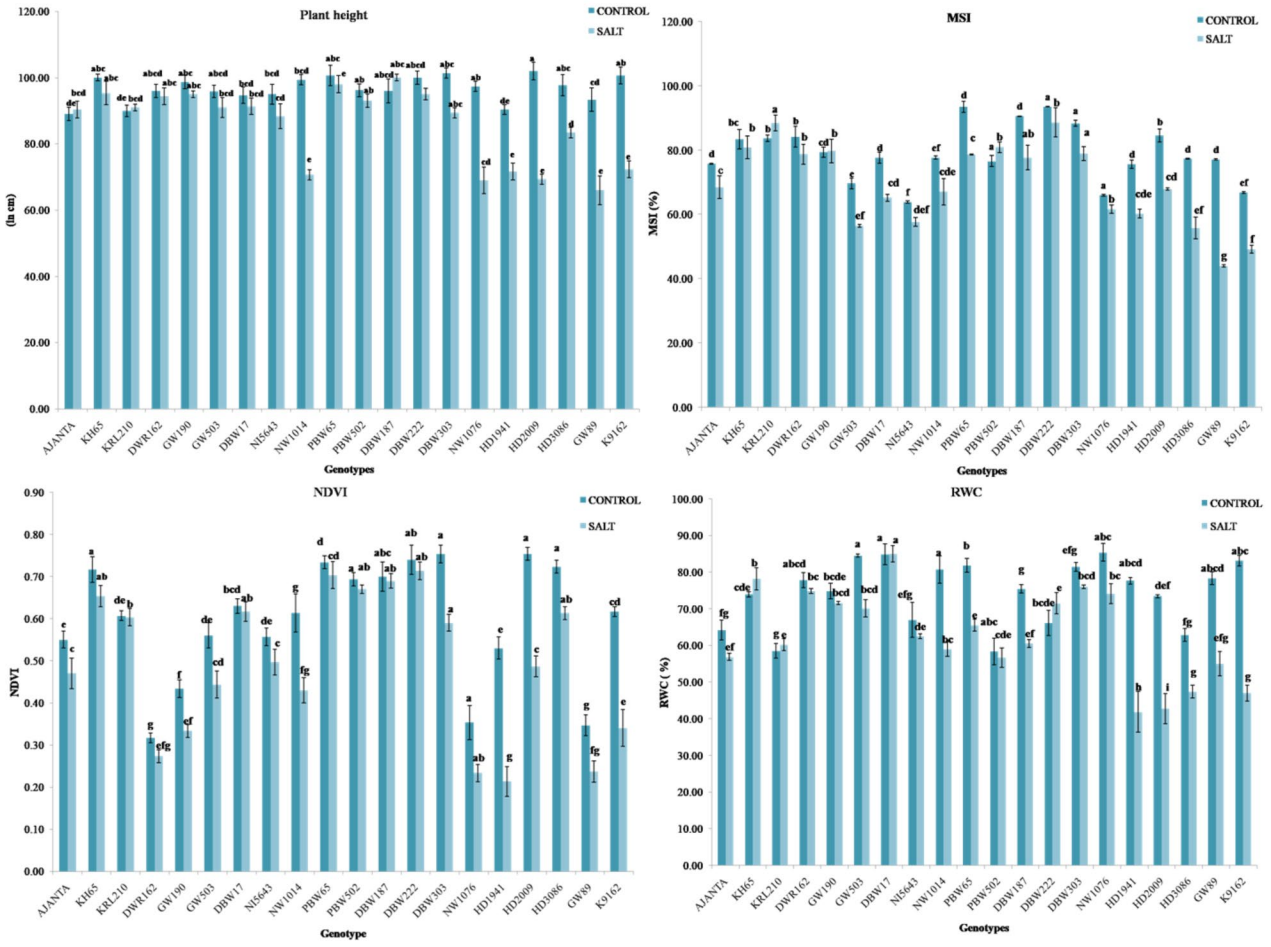

Fig. 1 Effect of salt stress on morpho-physiological parameters plant height, MSI (membrane stability index), NDVI (normal difference vegetation index), RWC (relative water content) in different cultivars of wheat. Values are expressed as means ± SD ($n = 3$) significant difference at $p \leq 0.05$

Antioxidant enzymes

SOD activity increased significantly at $p \leq 0.05$ in response to salt stress in all the wheat genotypes. The highest SOD activity was observed in KRL210 (30.70 units/g FW), and the lowest activity was observed in HD2009 (14.68 units/g FW). An increase in percent change was observed in the tolerant varieties KRL210 (24.63%) and GW190 (24.62%), whereas a decrease in percent change was observed in the sensitive varieties K9162 (-32.17%) and HD2009 (-28.62%) compared to the control under salt stress conditions (Supplementary Table 4, Fig. 4). Glutathione (GR) activity was also lower in the sensitive variety than in the tolerant variety under salt stress. The highest GR activity was observed in KRL210 (15.51 mM TNB min/g FW), and the lowest was observed in HD2009 (2.29 mM TNB min/g FW). The highest percent decrease in glutathione activity was detected in the sensitive varieties K9162 (-78.28%) and HD1941 (-68.44%), and the highest percent increase in glutathione activity was detected in KRL210 (15.13%) (Supplementary Table 4, Fig. 4). Similar results were also observed for CAT and APX under salt stress. The highest CAT activity was observed in KRL210 (26.49 µmoles/min/g FW), and the lowest activity was observed in HD2009 (9.10 µmoles/min/g FW) under salt stress. The highest percent reduction in CAT activity was observed in the sensitive varieties HD2009 (-29.78%) and K9162 (-29.44%), whereas a significant percent increase ($p < 0.05$) was detected in the tolerant varieties KRL210 (66.92%) AJANTA (65.50%) and the unknown tolerance level varieties DBW222 (68.13%) and DBW187 (59.72%) compared with the control (Supplementary Table 4, Fig. 4). The maximum APX activity was observed in KRL210 (8.51 µmoles/min/g FW), and the minimum was observed in HD2009 (1.06 µmoles/min/g FW). APX activity was also found to decrease among sensitive varieties. The highest percent reduction was observed in HD2009 (75.00%) and GW89 (-54.65%), and the highest percent increase was observed in the tolerant varieties GW503 (141.67%) and KRL210 (111.11%) compared to the control under salt stress conditions (Supplementary Table 4, Fig. 4).

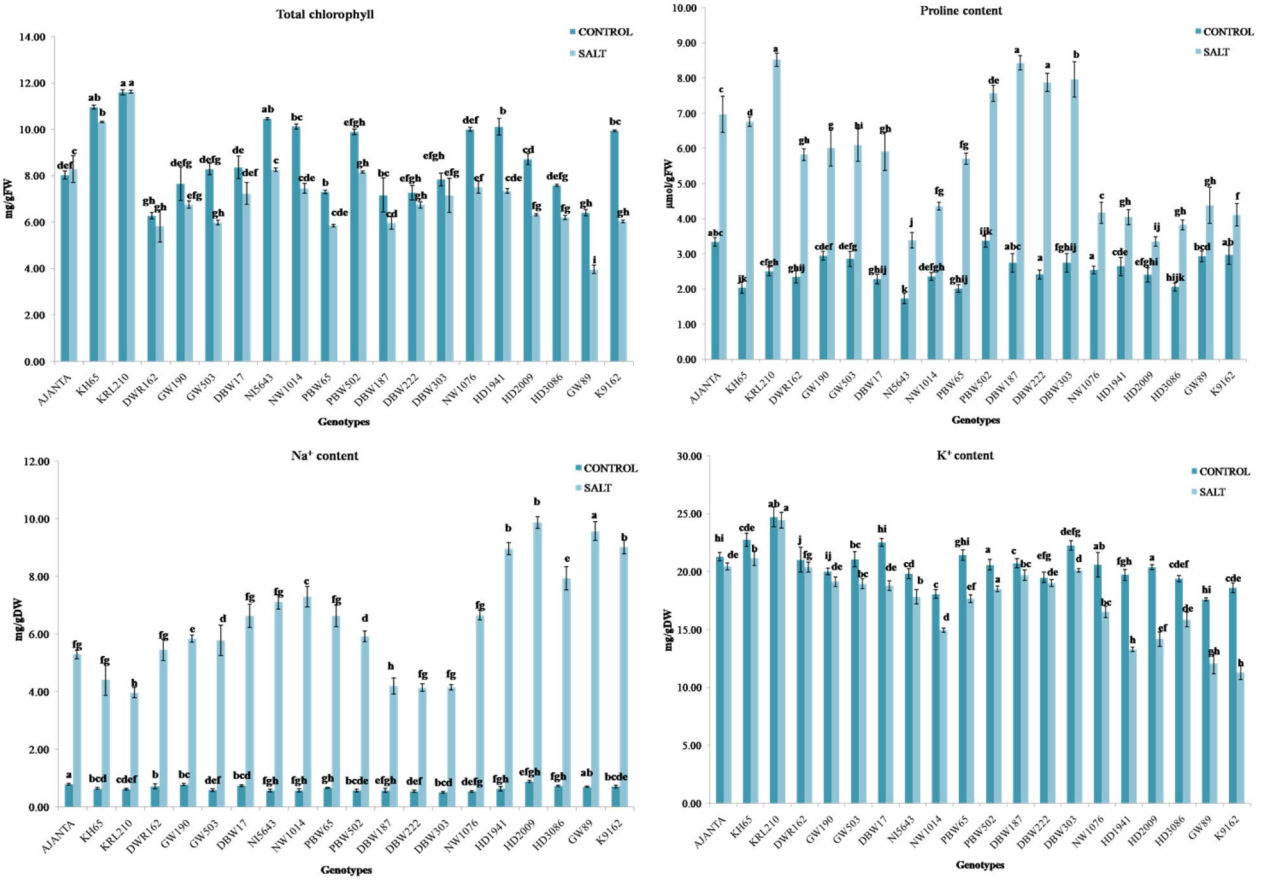

Fig. 2 Effect of salt stress on osmoprotectants total chlorophyll, proline, Na^+, and K^+ content in different cultivars of wheat. Values are expressed as means ± SD ($n = 3$) significant difference at $p \leq 0.05$

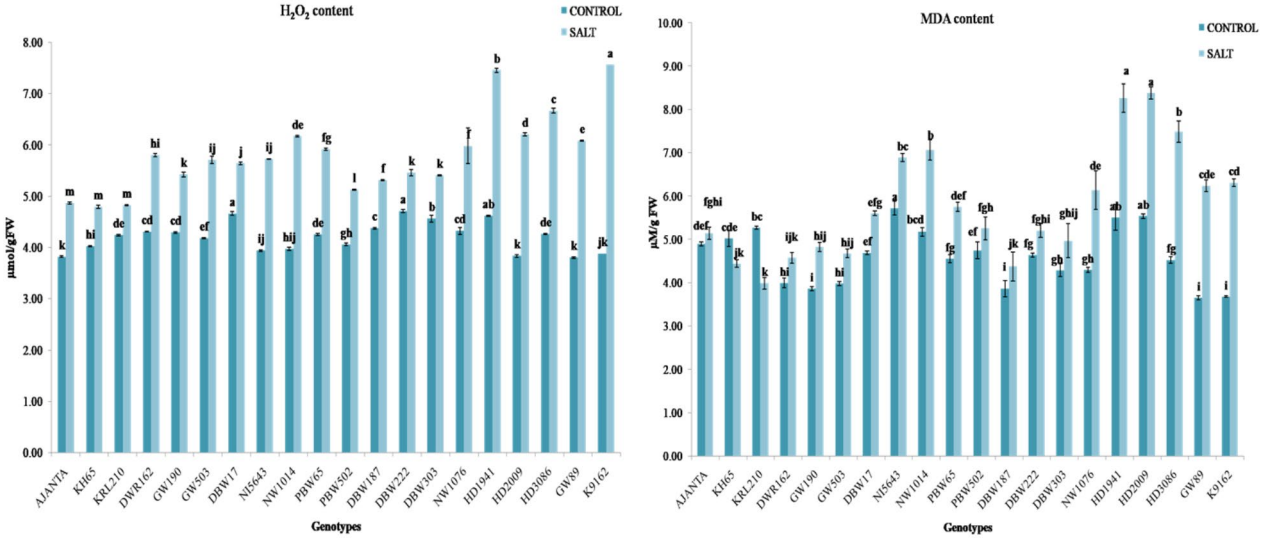

Fig. 3 Effect of salt stress on biochemical parameters H_2O_2 and MDA content in different cultivars of wheat. Values are expressed as means ± SD ($n = 3$) significant difference at $p \leq 0.05$

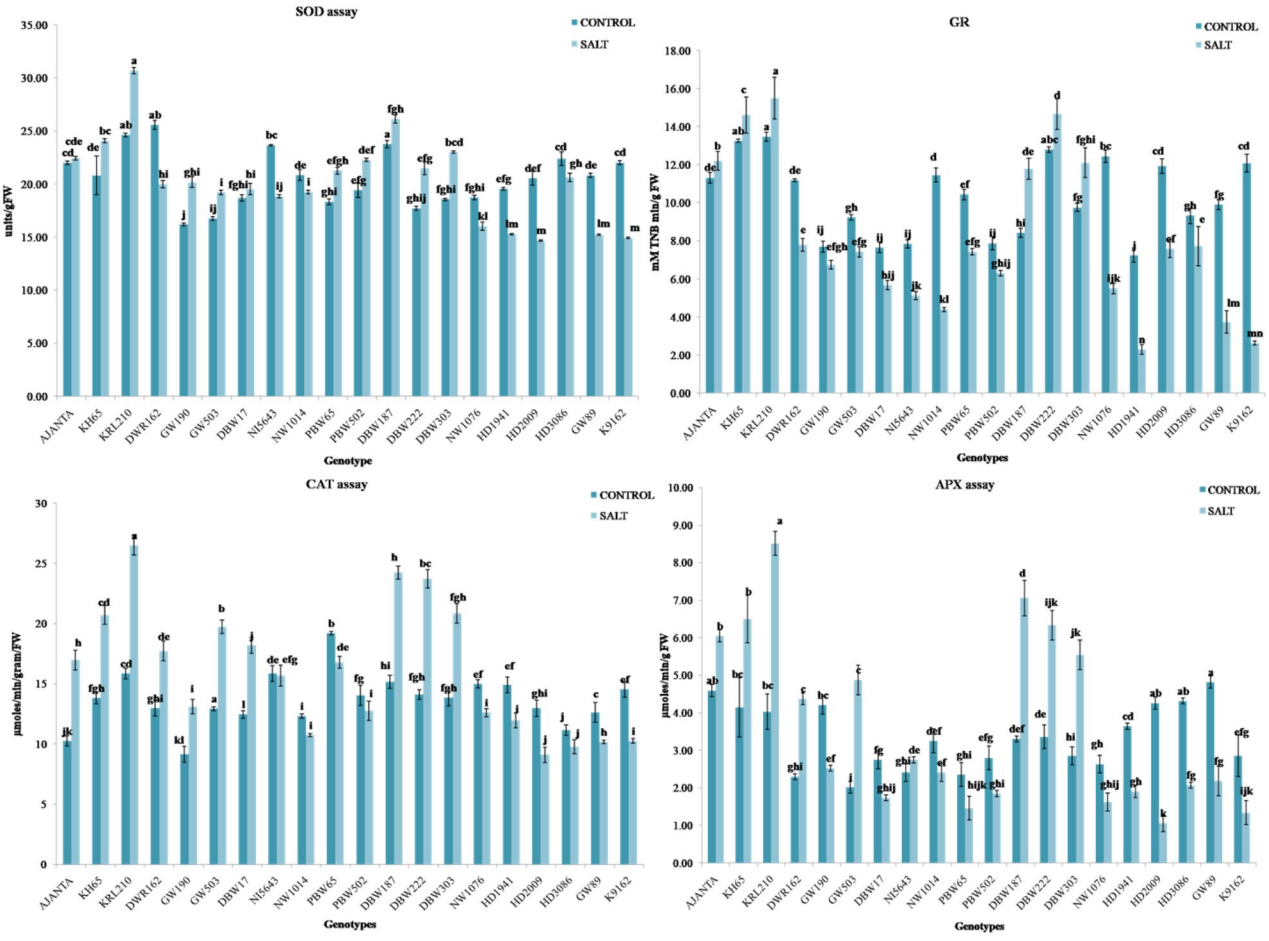

Fig. 4 Effect of salt stress on antioxidants parameters SOD (superoxide dismutase), GR (glutathione reducatse), CAT (catalase), APX (ascorbate peroxidase) in different cultivars of wheat. Values are expressed as means ± SD ($n = 3$) significant difference at $p \leq 0.05$

Na^+/K^+ accumulation

Salinity stress significantly ($p < 0.05$) increased the amount of Na^+ ions, decreased the K^+ content, and decreased the K^+/Na^+ ratio in the wheat cultivars. The maximum Na^+ content accumulation was observed in HD2009 (9.87 mg/gDW) and the minimum was observed in KRL210 (3.97 mg/gDW) under salt stress conditions (Supplementary Table 3, Fig. 2). A greater percentage increase in Na^+ was observed in the sensitive varieties HD1941 (1323.28%) and GW89 (1261.14%), whereas the tolerant varieties KRL210 (543.78%) and AJANTA (570.46%) exhibited a smaller percentage increase compared with the control under salt stress. Interestingly, the increase in Na^+ content among the cultivars was inversely proportional to the decrease in K^+ content. Under salt stress, a decrease in K^+ content was observed in all the wheat genotypes. The maximum K^+ content accumulation was observed in KRL210 (24.46 mg/gDW) and the minimum was observed in K9162 (11.29 mg/gDW) under salt stress conditions (Supplementary Table 3, Fig. 2). A large percentage reduction was observed in the sensitive varieties K9162 (-39.30%) and HD1941 (-32.55%) compared with the control, and the lowest percentage reduction was detected in the tolerant variety KRL210 (-1.12) and the unknown tolerance level variety DBW222 (-2.38%) compared with the control under salt stress conditions. A decrease in the K^+/Na^+ ratio was observed under salt stress conditions. The maximum K^+/Na^+ ratio accumulation was observed in KRL210 (6.16), and the minimum was observed in K9162 (1.25) under salt stress conditions (Supplementary Table 3).

Proline as an osmoprotectant

Exposure to salinity stress leads to the accumulation of osmolytes for better protection of cellular functions. Proline acts as an osmolyte under stress conditions. The maximum accumulation of proline was observed in KRL210 (8.52 μmol/gFW), and the minimum accumulation was detected in the sensitive variety HD2009 (3.36 μmol/gFW) under salt stress conditions. The highest percent increase was observed in the tolerant variety KRL210 (240.24%) and the unknown tolerance

level variety DBW222 (226.39%), and the least percent increase was observed in the sensitive varieties K9162 (38.38%) and HD2009 (39.56%) under salt stress conditions (Supplementary Table 3, Fig. 2).

Yield contributing factors

The sensitive genotypes exhibited a decrease in yield under salt stress conditions, while the tolerant and moderately tolerant cultivars maintained their photosynthetic ability; consequently, their yield was less affected by salinity. A decrease in spike length was observed in sensitive wheat cultivars under salinity stress. The maximum spike length was observed in KRL210 (13.77 cm), and the minimum spike length was observed in HD2009 (9.43 cm) under salt stress conditions. The highest percent reduction in spike length compared to the control was observed in K9162 (15.71%) and HD2009 (-14.24), while the greatest percent increase in spike length was observed in the tolerant varieties GW503 (20.63%) and KRL210 (18%) (Table 1).

GW/S decreased under salinity stress in sensitive wheat cultivars, while the maximum GW was observed in DBW187 (3.10 g) and the minimum was observed in HD1941 (0.66 gm) under salt stress conditions. The maximum percent reduction was observed in GW89 (-40.60%), HD2009 (-31.25%), and K9162 (-26.80), whereas the maximum percent increase was found in the tolerant cultivars DBW187 (33.26%) and DWR162 (19.88%) under salt stress compared to the control (Table 1). The TGW decreased under salinity stress in sensitive wheat cultivars, and the maximum TGW was observed in KH65 (39.21gm) and the minimum TGW was observed in GW89 (21.38 gm) under salt stress conditions. A higher percent reduction was detected in HD1941 (-24.64%), K9162 (-22.55%) and HD2009 (-22.50), whereas a percent increase was found in the tolerant variety KRL210 (11.57%) and unknown tolerance level variety DBW222 (9.57%) under salt stress. The highest reduction in thousand-grain weight was found in the sensitive varieties (Table 1).

Hectoliter weight (HW) decreased significantly ($p \leq 0.05$) in sensitive varieties. The Hectolitre weight is a measure of the volume of grain per unit. It is a good measure of grain quality and is used by millers globally as an indicator of expected flour yield. The maximum HW was observed in KH65 (76.1 g) and the minimum was observed in K9162 (58.05 gm) under salt stress conditions. The highest percent reduction was observed in GW89 (-17.37%) and HD1941 (-15.74%), whereas the highest percent increase was observed in the tolerant variety KRL210 (3.82%) (Table 1).

Salt stress is generally associated with an increase in quality-related parameters such as PC, gluten, and sedimentation value, but not moisture content. Therefore, the maximum protein content was observed in NI5643 (16.28%), the minimum was found in PBW65 (12.92%) under the control, the maximum was found in GW89 (16.67%), and the minimum was found in PBW65 (13.35%) under salt stress conditions (Table 1). Among the sensitive varieties, K9162 (12.15%), GW89 (11.92%), and HD2009 (11.85%) had the greatest increase in protein content, whereas less of an increase was observed among the tolerant variety GW503 (1.17%) and the unknown tolerance level variety DBW187 (1.30%). The maximum moisture content was observed in KRL210 (11.34%), the minimum moisture content was found in HD2009 (10.04%) under the control, the maximum moisture content was found in DWR162 (11.10%), and the minimum moisture content was found in K9162 (9.31%) under salt stress conditions. A decrease in moisture level was observed under salinity stress, and the greatest percentage reduction was observed in the sensitive varieties K9162 (-13.19%) and GW89 (-10.97%) compared to the control, while a smaller percentage reduction was found in DBW303 (-0.90%) (Table 1).

A sedimentation test was performed, and an increase in all varieties was observed under salinity stress. The maximum sedimentation value was observed in DBW187 (67.78 ml), and the minimum sedimentation value was found in PBW502 (40.25 ml) under the control, while the maximum sedimentation value was found in K9162 (65.49 ml), and the minimum sedimentation value was found in PBW502 (47.18 ml) under salt stress conditions (Table 2). The greatest percent increase was observed for the sensitive varieties K9162 (44.09%) and GW89 (33.88%), and the least percent change was observed for the DBW187 (10.22%) and tolerant KRL210 (10.62%) and KH65 (12.22%) varieties (Table 2).

Gluten content

The gluten content was significantly affected by salinity stress. The maximum wet gluten content was observed in HD3086 (30.5%), the minimum was found in KH65 (26.4%) under the control conditions, and the maximum was found in GW89 (36.8%), while the minimum was found in KH65 (28.2%) under salt stress conditions (Table 2). The maximum dry gluten content was observed in HD1941 (11.1%), the minimum was found in KRL210 (9.1%) under the control conditions, the maximum was found in HD1941 (13.6%), and the minimum was found in KRL210 (9.7%) under salt stress conditions. The highest percentage change in wet gluten was found in GW89 (32.85%), and the least percentage change was found in DBW222 (5.78%) and KRL210 (6.74%) compared to the control under salt stress. The highest percent change in dry gluten was found in K9162 (30.30%), and the least percentage change was found in DBW222 (3.85%) and

Table 2 Changes in the quality parameters of 20 wheat genotypes exposed to salinity stress conditions. The values are expressed as the means ± SDs ($n = 2$). The figures in the brackets depict the relative percentage of change from the control

	Sedimentation (ml)		Wet gluten (%)		Dry gluten (%)	
Genotypes	Normal	Salt	Normal	Salt	Normal	Salt
AJANTA	45.55	53.82 (18.17)	29.1	31.7 (8.93)	9.7	10.9 (12.37)
KH65	57.67	63.56 (10.21)	26.4	28.2 (6.82)	9.4	10.2 (8.51)
KRL210	50.74	55.55 (9.48)	28.2	30.1 (6.74)	9.1	9.7 (6.59)
DWR162	40.83	46.89 (14.85)	29.8	32.6 (9.40)	9.8	11.2 (14.29)
GW190	48.34	56.32 (16.53)	28.8	32.9 (14.24)	10.4	11.2 (7.69)
GW503	49.01	57.27 (16.85)	27.6	30.45 (10.33)	10.1	11.7 (15.84)
DBW17	52.09	62.00 (19.03)	29.9	32.3 (8.03)	10.6	11.7 (10.38)
NI5643	44.39	52.09 (17.34)	29.7	35.3 (18.86)	10.2	11.9 (16.67)
NW1014	48.62	55.27 (13.66)	29.1	33 (13.40)	9.3	10.4 (11.83)
PBW65	43.14	51.03 (18.29)	30.2	32.3 (6.95)	10.3	11.2 (8.74)
PBW502	40.25	47.18 (17.21)	28.1	30.8 (9.61)	10.1	10.9 (7.92)
DBW187	60.08	65.08 (8.33)	29.6	32.1 (8.45)	9.9	11.4 (15.15)
DBW222	52.38	61.46 (17.34)	29.4	31.1 (5.78)	10.4	10.8 (3.85)
DBW303	52.96	60.56 (14.36)	29.3	32.01 (9.25)	10.7	11.5 (7.48)
NW1076	46.80	60.98 (30.31)	29.71	33.1 (11.41)	9.6	11.9 (23.96)
HD1941	44.87	59.92 (33.54)	28.7	35.9 (25.09)	11.1	13.6 (22.52)
HD2009	42.27	54.50 (28.91)	28.2	34.2 (21.28)	10.2	13.2 (29.41)
HD3086	43.52	63.73 (46.43)	30.5	34.4 (12.79)	10.6	12.5 (17.92)
GW89	43.33	58.79 (35.67)	27.7	36.8 (32.85)	10.4	12.8 (23.08)
K9162	45.45	65.49 (44.09)	29.3	36.2 (23.55)	9.9	12.9 (30.30)
Mean	47.61	57.56	28.96	32.77	10.09	11.58

KRL210 (6.59%) compared to the control under salt stress (Table 2).

Statistical analysis

Principal component analysis (PCA)

PCA based on a correlation matrix was performed to study the relationships among 20 different wheat genotypes and different parameters related to growth, physiological, biochemical, yield, and quality traits under both control and salt conditions. Biplot vectors are trait factor loadings for PC1 and PC2 of 25 measured traits. Under control conditions, PC1 and PC2 explained 32.58% of the total variance (Fig. 5), and under salt conditions, PC1 and PC2 explained 70.41% of the total variance (Fig. 5). This PCA revealed four groups of genotypes based upon their salt tolerance level and all the parameters measured. The first group consisted of the highly tolerant genotypes KH65 and KRL210 along with three varieties of unknown tolerance, namely, DBW187, DBW222, and DBW303. This group was characterized by high activity of the enzymes CAT, APX, GR, and SOD under salt stress. The second group also contained the tolerant varieties AJANTA, PBW502, GW503 and GW190. This group had a high RWC, chlorophyll content, HW and moisture content. The third group contained the moderately tolerant genotypes NW1076, NW1014, and NI5643, and these genotypes showed elevated Na^+ and protein contents. The fourth group contained the sensitive genotypes HD1941, HD2009, GW89, K9162, and HD3086. This group had high Na^+, H_2O_2, MDA and gluten contents. Under control conditions, Na^+ had a negative correlation with PC1 and a positive correlation with PC2. K^+ showed a positive correlation for both PC1 and PC2. Under salt conditions, Na^+ was negatively correlated with both PC1 and PC2, and K^+ was positively correlated with PC1 but negatively correlated with PC2 (Fig. 5).

Correlation analysis between different traits

Correlation analysis between morphophysiological, osmoprotectants, biochemical, yield, and quality-related parameters was performed under control and salinity stress conditions (Fig. 6). Under salt stress conditions, correlation analysis revealed a positive correlation between the Na^+ content and the MDA and H_2O_2 contents (r=0.84, and 0.78; p<0.05), whereas the K^+ content negatively correlated with the H_2O_2 content (r = -0.86; p<0.05). The Na^+ content was positively correlated with the wet gluten and dry gluten contents (r=0.81 and 0.80; p<0.05, respectively) under salt stress. A positive correlation was observed between the yield parameter GW/S and SOD (r=0.87; p<0.05) under salinity stress, indicating a synergistic relationship. A significant positive correlation ($p \leq 0.05$) was detected between the yield parameter GW/S and the proline content (r=0.82; p<0.05), and a negative correlation was detected between the GW/S and the Na^+ content (r = -0.80, p<0.05) under salinity stress, which indicates an antagonistic relationship (Fig. 6). K^+ content positively correlated with GW/S

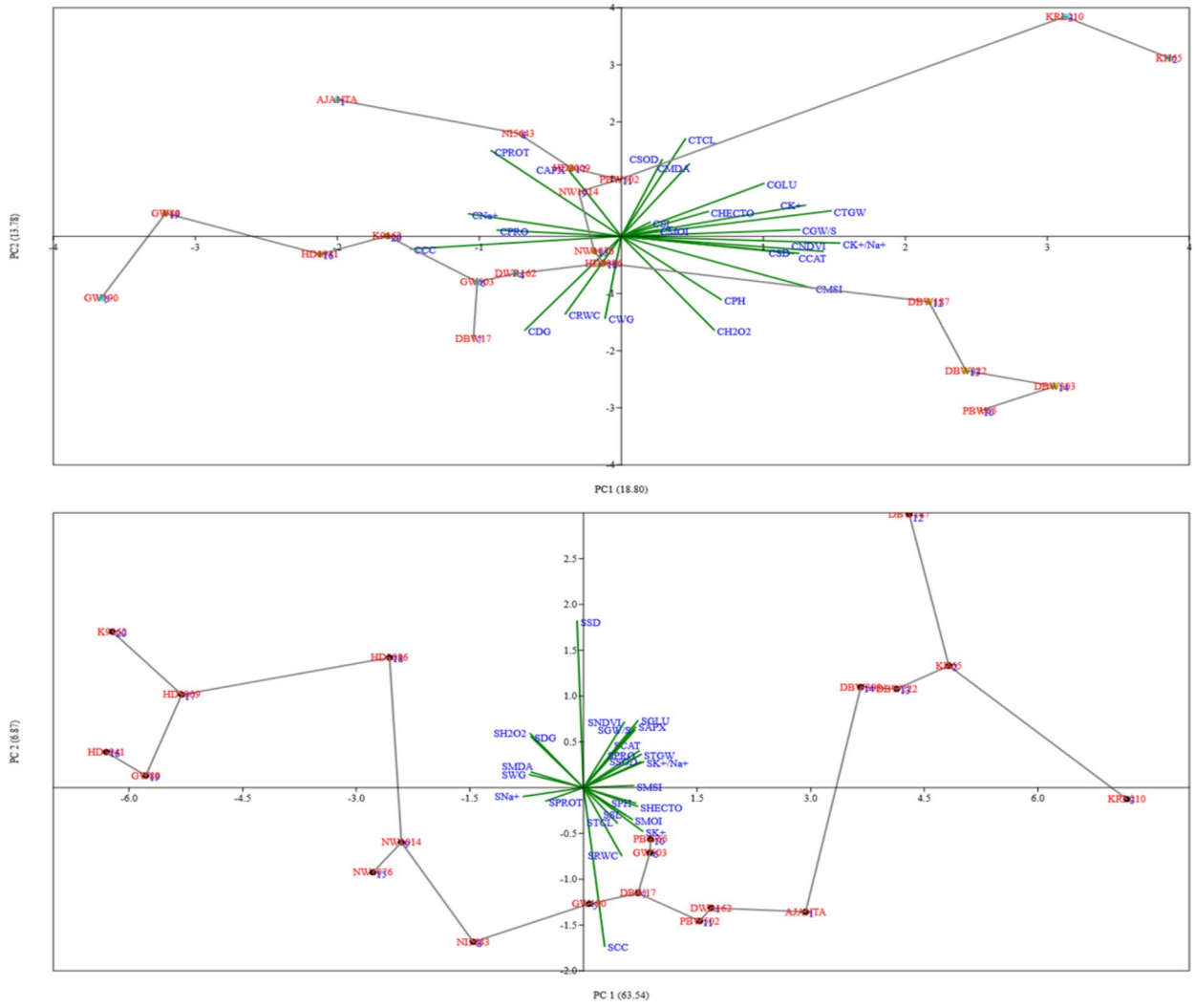

Fig. 5 Principal component analysis of 20 wheat genotypes under control and salt stress conditions. Biplot vectors are trait loading factors for PC1 and PC2 of different measured traits. Plant height (PH); NDVI; SPAD chlorophyll content; total chlorophyll content (CC); membrane stability index (MSI); relative water content (RWC); H_2O_2 content; MDA content; SOD, APX, CAT, and GR; Na^+ content; K^+ content; K^+/Na^+ ratio; proline content; spike length (SL); grain weight per spike (GW/S); TGW; hectoliter weight (HW); sedimentation test (SD); wet gluten (WG); and dry gluten (DG)

(r=0.71; p<0.05) under salinity stress. SOD, proline and K^+ exhibited a synergistic relationship with yield under salinity stress; conversely, Na^+ exhibited an antagonistic relationship with yield under salinity stress.

Discussion

The adverse effects of salinity on associated morpho-physiological, biochemical, and yield-related traits are researchable areas for the development of better-performing salt-tolerant genotypes. Large agricultural areas in arid and semiarid regions worldwide are affected by salinity, which is projected to worsen due to abrupt climatic change [17]. These findings suggest that several important physio-biochemical parameters are useful and effective for evaluating the salt tolerance of wheat cultivars and are good indicators of grain yield and related growth parameters under salinity stress. The loss of intracellular water content in plants is one of the main consequences of salinity. Water content is the key factor in the determination of growth and development in plants. Salinity stress decreases root hydraulic conductivity and reduces the water reuptake requirement from the soil, which in turn causes a significant reduction in leaf water content and leads to a decreased transpiration rate and photosynthetic efficiency [18, 19]. In the present study, compared with the moderately tolerant and tolerant cultivars, the moderately sensitive NW1014, NI5643 and sensitive cultivars K9162, HD2009, HD1941, and GW89 exhibited significant reductions in RWC, MSI, NDVI, and chlorophyll content. Compared with the salt-sensitive cultivar, the salt-tolerant wheat cultivar had greater leaf water potential and RWC, which is positively associated

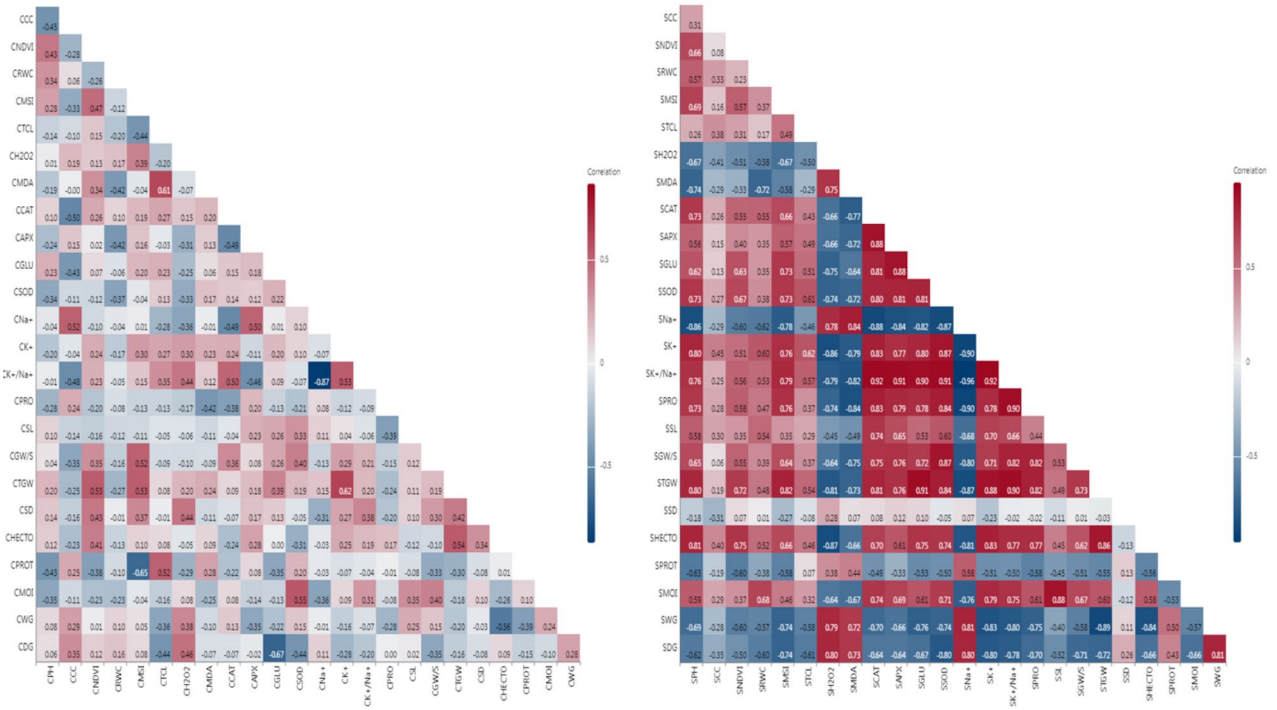

Fig. 6 Correlation analysis of wheat based on morpho-physiological, biochemical, yield, and quality-related parameters under control and salt stress conditions at a significance level less than ≤ 0.05. Red indicates positive values, and blue indicates negative values

with the accumulation of more osmoprotectants, indicating that the tolerant cultivar underwent effective osmotic adjustment. Sensitive cultivars are less capable of adjusting osmotically under salt stress conditions as supported by several previous studies [19, 20]. Munns [21] reported that a reduction in plant water status under salinity stress is the major cause of growth reduction. The tolerant varieties KRL210 and KH65 and of unknown tolerance varieties DBW187 and DBW222 displayed improved performance under salinity stress compared to the control. The results suggested that a greater reduction in photosynthetic pigments in sensitive varieties resulted in a decrease in growth and yield under salt stress. Na^+ ions toxicity under salinity do not cause degradation of chlorophyll molecules, instead Cl^- ions and high Na^+ were found to increase the chlorophyll content in faba beans [22]. In the present study, similar patterns of increase in chlorophyll content in tolerant cultivar KRL210 supports this observation [22].

Overproduction of ROS, including H_2O_2, O_2- and OH^-, as a result of salinity stress leads to the degradation of chlorophyll pigments, which are harmful oxidative markers [23]. Salinity treatment was observed to increase the generation of H_2O_2 and free radicals, causing significant damage to membrane lipids and loss of the membrane stability index. To prevent the oxidative damage that is triggered by ROS, plants intensify ROS-scavenging mechanisms. In the present study, salt stress elevated the level of H_2O_2. It has been reported that increased accumulation of ROS enhances antioxidant defense mechanisms, particularly in tolerant varieties [10]. MDA is one of the final products of the peroxidation of polyunsaturated fatty acids in the cell membrane. An increase in free radicals causes the overproduction of MDA, which is commonly known as a marker of oxidative stress and is used for evaluating plant tolerance or sensitivity to salt stress. MDA and electrolyte leakage (EL) diminish cell membrane integrity, cellular water content, and metabolic functions under salinity stress conditions. In this study, less accumulation of MDA was observed in the tolerant variety, and greater accumulation was observed in the sensitive wheat cultivars. Feki [24] reported that tolerance in wheat was associated with decreased accumulation of MDA and increased activity of antioxidant enzymes, which was substantiated by our results.

Plants utilize a complex antioxidant defense system to mitigate salt stress-induced damage [25]. CAT acts as a primary cellular H_2O_2 scavenging system by converting H_2O_2 to water and oxygen [6]. SOD is considered the main intracellular enzymatic antioxidant because it provides the first line of defense against ROS toxicity [6]. In the present study, the antioxidant activities of the enzymes SOD, CAT, APX, and GR were observed higher in tolerant, unknown tolerance level, and moderately tolerant wheat genotypes than in sensitive cultivars. The results indicate that the ability of the genotypes to utilize

their antioxidant system plays an important role in limiting the damage caused by salinity stress [13, 26]. It was reported that CAT and APX work in coordination with SOD activity to counteract the negative effects of O_2^- and $H_2O_2^-$ generated by salinity stress, which was also confirmed in this study [27]. To obtain electrons from the photosynthetic electron transport chain and reduce the production of ROS, increasing GR activity might increase $NADP^+$ concentrations [28]. Furthermore, our study implies that salt-tolerant genotypes might have a more active ascorbate-glutathione cycle.

A greater accumulation of Na^+ in plants in response to salt stress interferes with the accumulation of K^+ and further affects stomatal regulation. Previous studies have shown that the accumulation of Na^+ due to salt stress reduces the K^+ and chlorophyll contents in plants, which further leads to a decrease in growth [29]. Tolerant cultivars displayed improved K^+/Na^+ ratio maintenance under salt stress. The soluble sugar content, proline content, chlorophyll content, and $Na^{+/}K^+$ ratio significantly change under salinity stress, as they are important indicators of salt tolerance, as observed in *Catharanthus roseus* [30], *Brassica oleracea* [31] and *Zea mays* [32]. The accumulation of osmoprotectants in plant cells is an important mechanism for preserving the osmotic balance for the maintenance of physiochemical processes in cells. Salt stress increased proline accumulation, which subsequently improved photosynthetic efficiency, ATP production, and water use efficiency. Proline is a non-enzymatic antioxidant and plays a vital role in osmotic adjustment under salinity stress. Reducing the amount of ROS generated as a result of salt stress decreases the harmful effects of ROS and increases plant tolerance. Moreover, proline improves plant antioxidant systems. Proline accumulation assisted plants in recompensing energy and increased their survival under salinity stress [33]. The proline content is considered an optimal indicator for recognizing salinity stress, particularly at high salinity levels. The current study demonstrated that the proline concentration in the leaves of wheat plants under salinity stress increased considerably compared with that in the leaves of non stressed plants. The maximum concentration of proline was detected in the tolerant wheat cultivar KRL210 and the unknown tolerance level variety DBW187 under salt stress. Among the three varieties, DBW 187, DBW 222 and DBW 303 exhibited the same behaviour for traits associated with salinity tolerance as did the tolerant genotypes; hence, DBW 187, DBW 222 and DBW 303 can be considered tolerant, which was further corroborated through PCA of the traits.

The reduction in GW in our findings is consistent with those of others who have shown decreased weight of grains in wheat plants exposed to salt stress. The GW and its alterations in response to salt stress were lower in tolerant, of unknown tolerance and moderate varieties than in sensitive wheat cultivars. Under saline conditions, all glycophytes generally exhibit yield losses due to altered water and nutritional balance, a lower source-to-sink ratio, and inefficient photosynthetic efficiency [34]. Salt stress is known to affect plant height and spike length in wheat, where increasing salinity significantly reduces spike length [35]. The observed yield loss due to high salt stress during the reproduction and grain filling periods is in agreement with previous reports on wheat. A greater reduction in grain weight under saline conditions resulted in a considerable decrease in grain yield per plant [36]. Salt-tolerant wheat cultivars with lower Na^+ contents produced higher grain and biological yields under saline conditions. The reduction in grain yield under salt stress might have resulted from several causes, such as loss of photosynthetic capacity because of the effects of salinity on leaf development or longevity or the reduction in fertilization via a reduction in pollen viability and/or stigma receptivity.

Salt stress was associated with an increase in quality-related traits, such as protein content and sedimentation value, but not moisture content. Salinity reduces yield primarily by causing severe reductions in spike number, grain number, and 1,000-grain weight in wheat [37]. Salinity stress leads to an increase in protein content and a decrease in protein quality. Increased PC due to other abiotic stresses, such as heat stress, has also been noted by other researchers [38, 39], revealing that heat-induced grain weight loss is more significant than the loss of protein accumulation [40]. This finding is consistent with the observation that heat-sensitive genotypes possessed a much lower yield than other genotypes under heat stress conditions and, correspondingly, had a much greater PC in their grains. An increase in PC due to high temperatures during the grain-filling period can be partly explained by altered source-to-sink carbon partitioning and thereby interactions with metabolism and partitioning of N in sources and sinks [40]. In previous studies, it was reported that wet gluten and dry gluten contents, sodium dodecyl sulfate (SDS) sedimentation volume, and grain protein content significantly increased as a result of salt and drought stress. Previous studies reported an increase in PC with increasing salt stress levels, suggesting positive implications in terms of gluten-forming gene expression. Salt stress leads to higher gluten content in the salt-stressed plants as compared to control [41, 42]. Both saline and drought stress conditions resulted in considerable reductions in thousand-grain weight, grain protein yield, and test weight [42]. PCA analysis was performed to study the relationship between morpho-physiological, biochemical, yield and quality related parameters under control and salt stress condition. PCA analysis showed that in tolerant varieties KH65,

KRL210, and DBW222 demonstrated increased osmoprotectant proline, $K^{\pm}/Na^{\pm}$ ratio and antioxidants like SOD, CAT, APX under salt stress conditions which was also observed in several previous studies [43]. Grain yield (GY) was positively and strongly correlated, with each of spikes/plant, thousand-grain weight, spike weight, grains /spike, spike length and spikelets/spike under salinity as found in other reports [44].

Conclusion

Wheat cultivars with different genetic backgrounds showed diverse responses to salinity stress, which helped to identify key morpho-physiological, biochemical, yield, and quality-related parameters governing the anticipated salt tolerance. The MDA content, MSI, nonenzymatic osmolyte concentration, antioxidant enzyme activity, and toxic ion uptake significantly increased with increasing salinity. The salt-tolerant and moderately salt-tolerant genotypes showed evidence of possessing a more efficient mechanism against salt stress by protecting themselves from ion toxicity and osmotic injury and maintaining higher contents of K^+, photosynthetic pigments, and non-enzymatic osmolytes and greater antioxidant enzyme activity under salinity stress than in salt-sensitive genotypes. The proline content, K^+ content, and antioxidants parameters CAT, APX, SOD, and GR were found to be positively correlated with the yield-related parameters SL, GW/S, HW, and TGW under salinity stress. The unknown tolerance level varieties DBW187, DBW303, and DBW222 showed better adaptation to salinity stress due to their effective antioxidant system and higher proline and K^+ contents in line with those of the tolerant varieties. These varieties can be effectively used for the further development of salinity-tolerant lines and can also be cultivated in saline-sodic areas. Correlation analysis of parameters under salinity stress showed that the SOD and proline parameters were strongly positively correlated and that the Na^+ content was strongly negatively correlated with the grain weight per spike. Hence, these three factors could serve as effective criteria for the rapid screening of germplasms against salinity stress for the identification of new elite lines in breeding programs.

Materials and methodology

A total of 20 wheat cultivars tolerant, moderate, sensitive, and unknown tolerance, varieties were used in this study (Supplementary Table 1). All healthy seeds were procured from the Germplasm Resource Unit (GRU), Indian Institute of Wheat and Barley Research (IIWBR), Karnal, India. The study was conducted at the IIWBR during the 2021–2022 and 2022–2023 seasons. The field experiment was conducted using a randomized complete block design (RCBD) with three rows in each growing season. Each microplot was 3×6×1.5 m in size and had a transparent sheet of rain cover. The two microplots used for this study were as follows: (1) a control with normal soil (pH 8.0, EC 3.9) and (2) saline-sodic (pH 9.4, EC 4.02) soil. Microplot with sodic conditions were developed by adding the required quantity of sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) to the soil. The different wheat cultivars were planted in the third week of November. The crop was irrigated normally, and fertilizer was applied as per recommended agronomic practices (120 kg N, 60 kg P_2O_5, and 40 kg K_2O per ha) with a full dosage of P_2O_5 of 30 kg/ha and K_2O of 20 kg/ha at the time of sowing and N in three split doses of 60 kg/ha. Various morpho-physiological parameters, namely, chlorophyll content, NDVI, proline content, and Na^+/K^+ content, and biochemical parameters, such as H_2O_2, MDA, GR, SOD, CAT, and APX, were analysed at the flag leaf stage after heading, and fresh leaf samples were used for analysis of these experiments. Yield-related parameters, namely, plant height (PH), spike length (LS), grain weight per spike (GW/S), thousand-grain weight (TGW), and hectoliter weight (HW), were estimated at the maturity stage. All the chemicals used were of the highest quality and were procured from Sigma–Aldrich unless otherwise mentioned.

Physiological parameters

The chlorophyll content was measured at the flag leaf stage using a chlorophyll meter (SPAD-502, Minolta, Japan) for all the genotypes under both control and stress conditions. Three biological replicates and three technical replicates from both the control and stress conditions were selected, and SPAD values were recorded from the fully mature leaves counted from the top of the plants. The normalized difference vegetation index (NDVI) was measured at the flag leaf stage using the Crop Circle model ACS-430 from Holland Scientific (Lincoln, NE, USA) at 1.5 months from 0.5 m above the canopy. The membrane stability index (MSI) was estimated by measuring the electrical conductivity of the leaf leachates in DDW. The method of [45] was used to determine electrolyte leakage. Leaves were cut into small pieces and placed in a test tube containing 20 ml of deionized water. The electrical conductivity (EC)1 was determined after shaking the tubes for 4 h at 30 °C. The test tubes were then autoclaved at 121 °C for 20 min and cooled to 25 °C before the electrical conductivity (EC2) was measured. The following formula was used to determine electrolyte leakage (EC): EC1/EC2*100.

Hiscox and Israelstam's [46] method was used to determine the total chlorophyll concentration by using dimethyl sulfoxide (DMSO) as a solubilizing reagent. Briefly, leaf discs 1 cm in diameter were weighed (g) and placed in a test tube with 5 ml of DMSO. The test tubes were then placed in an oven for approximately 4 h at

60°C to facilitate the extraction of chlorophyll pigments. After reaching room temperature (2 hours), the O.D. was recorded at 2 different wavelengths, i.e., 645 and 665 nm, by using a UV–VIS spectrophotometer (Thermo Scientific, Japan). Welburn's [47] equations were used to determine the different pigments. The concentrations of all the pigments were calculated in mg g^{-1} tissue fresh weight. Chl 'a' (µg/ml)=12.19 A665–3.45 A645 Chl 'b' (µg/ml)=21.99 A645–3.32 A665 Total chlorophyll=Chl 'a' + Chl 'b'.

Antioxidant parameters

Determination of H_2O_2 content

The concentration of H_2O_2 was estimated by extracting fresh leaf samples at the flag leaf stage in 0.1% (w/v) trichloroacetic acid (TCA) using a mortar and pestle. After centrifugation at 12,000× g for 15 min, a known volume of the supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (1 mL). Subsequently, the optical density of the mixture was measured at 390 nm, and the results are expressed as µmol g-1 FW using the extinction coefficient of 0.28 µM-1 cm^{-1} H_2O_2 [48].

Determination of MDA content

Lipid peroxidation was measured by estimating the MDA content [49]. For lipid peroxidation determination, fresh leaves were extracted in 1%, w/v, TCA. After centrifugation at 10,000× g for 5 min, 1.0 mL of the supernatant was mixed with 0.5% thiobarbituric acid (TBA), and the mixture was boiled at 95 °C for half an hour. After that, the tubes were kept in an ice bath, followed by centrifugation for 5 min at 5000× g for clarification, and the optical density was measured at 532 nm and 600 nm. The MDA concentration was determined by dividing the difference in absorbance (A532–A600) by its molar extinction coefficient (155 mM^{-1} cm^{-1}), and the results are expressed as mmol g^{-1} fresh weight.

Enzymatic assays

The activity of the enzyme APX was assayed according to the protocol of [50]. The 3 ml reaction mixture was composed of 2.8 ml of 50 mM potassium phosphate buffer (pH 7.0), 15 µl of 0.5 mM ascorbic acid, 30 µl of tissue extract, and 155 µl of 0.5 mM H_2O_2. The decrease in absorbance at 290 nm was recorded, and an extinction coefficient of 2.8 mM^{-1} cm^{-1} for reduced ascorbate was used to calculate the enzyme activity. The specific enzyme activity was expressed as µmol mg^{-1} protein min^{-1}. SOD activity was assayed using the method of [51]. For the measurement of SOD activity, 100 µl of supernatant and 400 µl of riboflavin (1 mM) were mixed with 2.5 ml of assay mixture comprising 50 mM phosphate buffer (pH 7.8) supplemented with (2 mM) EDTA, (9 mM) L-Met, (50 µM) NBT and 0.025% Triton X100. The reaction was initiated by illuminating the samples for 10 min, followed by recording the absorbance at 560 nm instantaneously after the reaction stopped. The enzyme activity (gFW^{-1}) was estimated from the standard curve using pure SOD. The irradiation of the samples was performed under 20 W fluorescent light lamps for 40 min. The absorbance of this irradiated solution was measured at 560 nm with a spectrophotometer (Double Beam Spectrophotometer-UV 3000+, Lab-India Analytical). The enzyme activity of CAT was estimated based on H_2O_2 decomposition at a wavelength of 240 nm for 5 min according to the method of [52]. The reaction mixture (1.5 mL) was composed of 50 mM potassium phosphate buffer (pH 7.0), EDTA (0.1 mM), H_2O_2 (30%), and the enzyme extract (100 µL). The reduction in the OD was followed up to 5 min, and 1 unit of CAT was defined as 1 mmol H_2O_2 mL^{-m} min^{-m}. GR activity was assayed by recording the increase in absorbance in the presence of oxidized glutathione (GSSG) and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) [53]. For GR determination, a 3 ml assay mixture comprising 100 µl of supernatant, 1.82 ml of potassium phosphate buffer, 50 mM EDTA (2 mM), 750 µl of DTNB (0.75 mM), 30 µl of NADPH (0.1 mM), and 300 µl of GSSG (1 mM) (oxidized glutathione) was used. The reaction was initiated by the addition of GSSG, and the increase in absorbance at 412 nm was recorded for 5 min. The GR activity was calculated using the extinction coefficient of TNB (14.15 m–1 cm^{-1}) and expressed in terms of mM TNB min/gFW [53].

Na^+/K^+ estimation

Twenty-one-day-old flag leaves were used for the evaluation of Na^+ and K^+. One hundred-milligram flag leaf samples were dried for 48 h at 65 °C and digested with 0.5 ml of 0.5 N HNO_3 for 2 h at 80 °C [54]. The digested samples were centrifuged and diluted 100 times with distilled water. Concentrations of Na^+ and K^+ ions were measured by flame photometry using standards in the range of 0.25–20 ppm and expressed as milligrams per gram dry weight (mg/g DW).

Proline estimation

Proline was extracted from a 21-day-old flag leaf as previously described [55]. Fifty milligrams of fresh leaf sample was homogenized in 3% sulfosalicylic acid (5 µl/mg FW), kept on ice for 5 min, and centrifuged at 14,000 rpm for 10 min at room temperature, after which the resulting supernatant was used for determining the proline content. The reaction mixture containing 200 µl of glacial acetic acid, 200 µl of ninhydrin reagent, and 100 µl of supernatant was incubated for 20 min at 90 °C in a water bath. The reaction was terminated by transferring the reaction mixture tubes to ice. One milliliter of toluene

was added to the reaction mixture, and the mixture was vortexed. The upper toluene phase was taken for measurement of proline using absorbance at 520 nm. The proline content was measured against proline as the calibration standard and expressed in micromole per gram fresh weight (μmol/gFW).

Yield- and quality-related parameters

Spike length was estimated at the time of maturity just before harvesting using a standard scale. Spike length was estimated from its base to the tip excluding the awns and measured in cm. The grain weight per spike (GW/S) was estimated after harvesting. The TGW was determined using an automatic seed counter (Digital Seed Counter, Green Agritech), and an average weight of grain samples from two years for both the control and salt stress conditions was taken from each plot.

Hectoliter weight (HW)

The HW was determined using a hectometer (Indigenous test weight instrument, IIWBR) and measured as kg/hl for both control and salt stress conditions [56].

Protein and moisture content

The grain protein content and grain moisture content were determined by near-infrared reflectance spectroscopy (NIR, Analyser Inframatic 8620, FOSS) for both the control and under salt conditions.

Sedimentation test

A sedimentation test is used to measure gluten strength among different wheat genotypes. The SDS sedimentation test measures the sedimentation value of the suspension of flour in an SDS-lactic acid solution. The sedimentation value depends mainly upon the amount and swelling characteristics of glutenin. The sedimentation test was performed according to the standard protocol of the IIWBR [56].

Gluten content

The gluten content of the whole meal flour was estimated using the gluten-washing method [57]. The wet gluten (WG) content was measured immediately after washing the flour with tap water, and the wet gluten obtained was dried in a hot air oven at 100 °C for 24 h, after which the dry gluten (DG) content was estimated.

Statistical analysis

The experiments were arranged in a randomized complete block design with three replications. The data obtained were analysed by ANOVA, and all means were separated at the $p<0.05$ level using Tukey's HSD test. Analysis was performed using STAR ver. 2.0.1 software. The data are presented as the mean±SD from three independent biological replications for each sample. Principal component analysis (PCA) was performed on various physiological, biochemical, yield, and quality-related parameters by using PAST software (version 4.06b), and PCA plots were generated using (principal component) PC1 and PC2. Correlation analysis was performed using Minitab software.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05508-4.

Supplementary Material 1

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Author contributions

NP, VP, AY, and SR planned and designed the experiments; NP performed plant growth experiments; NP and VP carried out data interpretation; NP and VP drafted the manuscript; NP, VP, OPG, AY, MRM, SR, and GS revised the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

All data generated or analysed during this study are included in this manuscript and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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