Article

Differential Morphophysiological, Biochemical, and Molecular Responses of Maize Hybrids to Salinity and Alkalinity Stresses

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Abstract: Salinity and alkalinity stresses are common in arid and semiarid climates. Both these stresses not only retard crop growth but also cause a severe reduction in yields. The present experiment was performed to investigate the morphological, physiological, biochemical, and genetic responses of two maize hybrids (FH-1231 and DK-6714) to salinity and alkalinity stresses. The treatments were comprised of salt stress (NaCl:Na₂SO₄ at a 9:1 ratio), alkaline stress (NaHCO₃:Na₂CO₃ at a 9:1 ratio), and an unstressed control. The results indicated that salinity and alkalinity significantly reduced shoot fresh weight by 50% and 70%, root fresh weight by 38% and 50%, root dry weight by 69% and 93%, seedling length by 18% and 30%, number of leaves by 27% and 39%, and maximum leaf width by 17% and 24%, respectively, across the two hybrids compared with control, indicating that alkalinity had a greater effect than salinity. Likewise, both the stresses, particularly alkalinity, significantly decreased K⁺ ion accumulation and chlorophyll content and increased the lipid peroxidation rate, sodium (Na⁺) concentration, the hydrogen peroxide (H₂O₂) level, and the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). Salinity and alkalinity stresses also induced the expression levels of antioxidant genes (SOD, CAT, POD, APX); however, salinity showed less effect than alkalinity stress. Similarly, hybrid DK-6714 performed comparatively better than FH-1231 with regard to seedling growth, antioxidant activities, and biochemical attributes under stress conditions. Thus, DK-6714 is recommended as a suitable hybrid for soils affected with salt-alkalization.

Keywords: alkalinity; antioxidants; gene expression; ionic balance; maize hybrids; stress tolerance

1. Introduction

The occurrence of salinity and alkalinity stress is a major issue in arid and semiarid areas, where rainfall is insufficient for better plant growth [1]. These stresses can adversely affect plant growth and production [2]. Maize (*Zea mays* L.) is known as the queen of cereals and is grown in more than 116 countries due to its high yield potential and versatility [3]. It ranks third in the world after wheat and rice in terms of cultivation area, while in Pakistan, it is the fourth major crop after wheat, cotton, and rice [4]. The maize crop is grown between 50° north latitude to 40° south latitude, where biotic and abiotic stresses are commonly found [5]. Various abiotic stresses (such as waterlogging, drought, salinity, high and low

temperatures, and nutrient stress) negatively affect maize yield [6]. In particular, maize is a quite sensitive crop to salt stress [7].

Salinity and alkalinity are two different types of stresses that restrict plant growth [8]. Salinity is caused by neutral salts such as NaCl and Na₂SO₄, while alkalinity is caused by alkaline salts such as NaHCO₃ and Na₂CO₃ [9]. It is estimated that about 932 mha of land worldwide is affected by salt-alkalization [10,11]. In Pakistan, the majority of the soils contain low organic matter and are alkaline in nature, with pH $\geq$ 8, due to a high concentration of calcium carbonate, and the salt-affected area in Pakistan is 6.30 mha [12,13].

Both salinity and alkalinity stresses are the major limiting factors for plant growth and development [2]. The most common morphological symptom of salinity stress on plants is decreased growth, which is the result of many physiological responses [14,15]. Soil alkalization mostly occurs simultaneously with soil salinization [16,17]. Alkalinity has similar effects to soil salinity and has an additional effect due to high pH [18,19]. A high concentration of alkaline salts reduces growth and biomass accumulation in plants [20]. Under neutral and alkaline salt stress, chlorophyll content and the relative growth rate of root and shoot were decreased, but such decrease was greater under alkaline salt stress [21].

Salinity and alkalinity severely interfere with the ion uptake mechanism. The excessive accumulation of Na⁺ ions under salt stress reduces the uptake of K⁺ ions [22,23]. Ionic toxicity disturbs the osmotic balance and leads to reduced plant growth [24]. Ion toxicity reduces growth and causes premature leaf senescence that leads to a reduction in photosynthetic pigments because leaves are an important photosynthetic area that converts light energy to potential energy through green pigments [25–27]. Under stress conditions, lipid peroxidation is also the result of specific ion toxicity or excessive accumulation of Na⁺ ions [28]. The malondialdehyde (MDA) content in *Populus cathayana* was increased with an increase in alkalinity stress, which indicates that a high level of soil alkalinity severely damages the membranes [23].

Both of these stresses trigger the production of reactive oxygen species (ROS) in plants [29]. Reactive oxygen species, such as singlet oxygen, superoxide radical, hydroxyl ion and hydrogen peroxide (H₂O₂), are produced in plants even under normal conditions during photosynthesis, respiration, and photorespiration, but plant cells maintain a balance between ROS and antioxidants [29–31]. However, an increase in synthesis and accumulation of ROS causes membrane denaturation by reacting with proteins, DNA, lipids, and other macromolecules [32]. Under the influence of saline and alkaline salts, the over-accumulation of ROS enhances the activity of antioxidant enzymes such as SOD, CAT, POD, and APX that are involved in the mitigation of ROS [33,34]. Although a number of studies have reported that neutral and alkaline salt stresses cause changes in the growth, physiology, and oxidative metabolism of crops, the effects of salt-alkalization on the growth and oxidative metabolism of maize are poorly understood. Therefore, the present study was conducted to evaluate the effects of salt-alkalization on the morphology, growth, chlorophyll content, reactive oxygen species, and antioxidant defense system of two maize hybrids.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Two maize hybrids, FH-1231 and DK-6714, were used for this experiment. In a preliminary experiment on germination and seedling growth, DK-6714 appeared moderately tolerant, while FH-1231 was sensitive to salt stress (data not shown). Both of these hybrids are commonly grown by the local maize farmers of Punjab-Pakistan. The seeds of FH-1231 and DK-6714 were collected from Ayub Agricultural Research Institute, Faisalabad, and Monsanto, Pakistan (Pvt.), respectively. The recommended dose of fertilizers (250:140:90 mg/pot N:P:K) was applied as a basal dose. Urea was used as a nitrogen source, while single super phosphate (SSP) and sulfate of potash (SOP) were used as the source of phosphorus and potassium, respectively. The experiment was carried out in the greenhouse of the Department of Agronomy, University of Agriculture Faisalabad. The experiment was

arranged in a completely randomized design under the factorial arrangement, with four replicates. The experiment contained 24 pots. In each pot, 15 seeds were sown at equal distances, and 5 seedlings were thinned out after emergence. The treatments included salt stress ($\text{NaCl}:\text{Na}_2\text{SO}_4$ with a ratio of 9:1), alkaline stress ($\text{NaHCO}_3:\text{Na}_2\text{CO}_3$ with a ratio of 9:1), and a no-stress control. For saline and alkaline stresses, electric conductivity (EC) was maintained at 8 dSm^{-1} , while the pH values of neutral and alkaline salts were 7.8 and 9.4, respectively.

2.2. Data Recorded

2.2.1. Morphological Growth

After 20 days of the treatment application, the plants were harvested. The shoot and root lengths were measured by using a meter rod. After the dissection of roots and shoots, their fresh and dry weights were measured by using an electric balance. To measure the dry weight of roots and shoots, the samples were sun-dried for three days and then oven-dried at 75°C until constant weight.

2.2.2. Photosynthetic Pigments

The chlorophyll contents in maize hybrids were determined according to the method of Xu et al. [35]. Briefly, fresh leaves were chopped into small pieces and added to 5 mL of 80% acetone. This solution was then filtered and centrifuged at 14,000 rpm and 4°C for 15 min. The absorbance of the sample was measured at wavelengths of 665, 649, and 470 nm for chlorophyll a, chlorophyll b, and carotenoids, respectively.

2.2.3. Inorganic Ion

The Na^+ and K^+ ion concentrations were measured according to the method of Williams and Twine [36]. The dried plant material was ground and treated with 2 mL of 80% perchloric acid and 10 mL of concentrated sulphuric acid for 12 h. The digested material was then diluted to a volume of 100 mL with distilled water, and the concentrations of Na^+ and K^+ were measured by a flame photometer.

2.2.4. Lipid Peroxidation and H_2O_2 Content

The H_2O_2 content was determined according to the method of Velikova et al. [37], while the lipid peroxidation rate was determined as the MDA content using the method of Heath and Packer [38]. A fresh leaf sample of 250 mg was frozen in liquid nitrogen, and then leaves were ground in trichloroacetic acid (TCA, 3 mL) and centrifuged at $13,000 \times g$ for 7 min at 4°C . Then, 2 mL of supernatant was mixed with 0.67% TCA (2 mL) and heated at 100°C for 30 min. Afterward, the mixture was centrifuged again at $12,000 \times g$ for 10 min, and the absorbance was recorded at 532 and 600 nm wavelengths. The blank sample of 0.025% thiobarbituric acid in 10% TCA was also run. The content of MDA was computed using the given formula and is showed in $\mu\text{mol g}^{-1} \text{FW}$:

$$\text{MDA} = (\text{A}_{532} - \text{A}_{600})/\epsilon$$

where, ϵ is the extinction coefficient.

2.2.5. Antioxidant Enzymes Activities

The activities of CAT and POD in maize leaves were determined according to the method of Chance and Maehly [39]. For CAT activity, 0.3 mL of enzyme solution, 0.3 mL of phosphate buffer (pH 7.8), and 4.5 mL of 100 mM H_2O_2 were added to a 10 mL test tube, and absorbance was measured at 240 nm at one-minute intervals for 4 min. To determine POD activity, a reaction mixture containing 50 mM of phosphate buffer (pH 7.0), 10 mM guaiacol, and 5 mM of H_2O_2 was prepared. The mixture was preheated at 25°C in a water bath, and then 0.3 mL enzyme solution and 2.8 mL of reaction solution were added to a 10 mL centrifuge tube and mixed thoroughly. The absorbance of the mixture was measured at 470 nm at one-minute intervals, and the measurement continued for 4 min. SOD activity

was measured according to the method of Dhindsa et al. [40]. Samples of fresh leaves (0.2 g) were homogenized in 2 mL of ice-cold, 0.1 M phosphate buffer of 7.8 pH, 0.1 mM EDTA, and 0.1% polyvinylpyrrolidone and centrifuged at 12,000 rpm and 4 °C for 15 min. In a 10 mL test tube, 0.2 mL extract solution, 1.6 mL phosphate buffer, 0.3 mL of 130 mmol/L Met buffer, 0.3 mL of 750 µmol/L NBT buffer, 0.3 mL of 100 µmol/L EDTA-Na₂ buffer, and 0.3 mL of 20 µmol/L lactoflavin were mixed, and then the absorbance was recorded. APX activity was determined according to the method of Nakano and Asada [41]. The reaction mixture was prepared by mixing 50 mM phosphate buffer (pH 7.0), 0.25 mM ascorbic acid, 1 mM H₂O₂, and 0.1 mM EDTA. In a 10 mL centrifuged tube, 2.8 mL reaction solution and 0.2 mL enzyme solution were mixed immediately, and the absorbance was measured at 290 nm at one-minute intervals; the measurement continued for 4 min.

2.2.6. RNA Isolation, cDNA Synthesis, and Transcriptional Analysis

Quantitative real-time PCR (qRT-PCR) analysis was carried out to assess the expression levels of the genes encoding antioxidant enzymes (*catalase* (CAT), *superoxide dismutase* (SOD), *peroxidase* (POD), and *ascorbate peroxidase* (APX)) in 20-day-old maize seedlings of the two hybrids (DK-6714 and FH-1231). Total RNA was extracted in liquid nitrogen from the fresh plant tissues, and cDNA was obtained using the RNeasy Plant Mini Kit and the Reverse Transcription Kit (Qiagen, Hilden, Germany), respectively. All reactions were done in triplicate in a final volume of 25 µL, following the manufacturer's protocol of the QuantiTect SYBR Green PCR Kit (Qiagen, Hilden, Germany). PCR amplification conditions were 95 °C for 10 min; 40 cycles of 95 °C for 20 s, 60 °C for 30 s, 72 °C for 2 min, and 72 °C for 4 min. A melt-curve analysis was assayed to verify the amplification specificity. Gene specific-primers for SOD and APX [42,43] as well as for CAT and POD [44] were obtained from the previous studies (Table 1). The *Actin2* gene was used as an internal reference [44], and the levels of the relative gene expression were determined by the 2^{-ΔΔCt} method [45].

Table 1. Primer sequences for qRT-PCR amplification of antioxidant genes.

Genes	Gene ID (Accession Number)	Primer Sequences (5'–3')	References
SOD (<i>superoxide dismutase 4</i>)	NM_001112234	F: TGGAGCACCAGAAGATGA R: CTCGTGTCC ACCCTTTCC	Vwioko et al. [42]; Neta et al. [43]
APX (<i>ascorbate peroxidase 2</i>)	NM_001112030	F: TGAGCGACCAGGACATTG R: GAGGGCTTTGTCACTTGGT	Vwioko et al. [42]; Neta et al. [43]
CAT (<i>catalase isomer 3</i>)	NM_001152957	F: AATCGCCTCACGAATCGGTCCT R: CGCACCACAACAACCACTACGA	Huang et al. [44]
POD (<i>peroxidase 39 isoform X1</i>)	NM_001112570	F: TCGGCGTCATCGTCAGTGCT R: GGCGAGGTTGAGGCTCTTGTTTC	Huang et al. [44]
<i>Actin2</i>	EU958048	F: TGAAACCTTCGAATGCCAG R: GATTGGAACCGTGTGGCTCA	Huang et al. [44]

2.3. Statistical Analysis

Data collected was analyzed using a two-factor factorial experiment under a completely randomized design (CRD), and the comparison among treatment means was done using a least significant difference (LSD) test at a probability level of 5%.

3. Results

3.1. Seedling Fresh and Dry Weights

Both salinity and alkalinity treatments significantly reduced seedling fresh and dry weights (Figure 1). Salinity stress reduced the shoot fresh weight of DK-6714 and FH-1231 by 49% and 50%, root fresh weight by 31% and 46%, shoot dry weight by 64% and 72%, and root dry weight by 68% and 70%, respectively, compared with control. Exposure to

alkalinity stress reduced the shoot fresh weight of DK-6714 and FH-1231 by 65% and 75%, root fresh weight by 49% and 50%, shoot dry weight by 81% and 86%, and root dry weight by 93% and 93%, respectively, over control. For all traits, the negative effects of alkalinity stress were more severe than salinity stress. Under stress conditions, DK-6714 performed better than FH-1231 and recorded less reduction in growth and biomass (Figure 1).

Figure 1. Effect of salinity and alkalinity stress on (A) shoot fresh weight, (B) root fresh weight, (C) shoot dry weight, and (D) root dry weight of 20-day-old maize hybrids (DK-6714 and FH-1231). The bars represent the standard error ($\pm$ SE) of the four replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

3.2. Seedling Length and Number of Leaves

Seedling length, number of leaves per seedling, and maximum leaf width differed significantly among salt treatments and maize hybrids (Figure 2). Salinity stress reduced the shoot length by 19% and 21%, root length by 15% and 16%, number of leaves by 27% and 27%, and maximum leaf width by 10% and 25% in DK-6714 and FH-1231, respectively, compared with control. Exposure of alkalinity stress reduced the shoot length by 29% and 32%, root length by 29% and 32%, number of leaves by 37% and 40%, and maximum leaf width by 14% and 33% in DK-6714 and FH-1231, respectively, over control, indicating that the negative effects of alkalinity stress were more severe than salinity stress. Under saline and alkaline stress conditions, DK-6714 showed less reduction in the above parameters compared with FH-1231 (Figure 2).

Figure 2. Effect of salinity and alkalinity stress on (A) shoot length, (B) root length, (C) number of leaves per seedling, and (D) maximum leaf width of 20-day-old maize seedlings of two hybrids (DK-6714 and FH-1231). The bars represent the standard error (SE) of the four replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

3.3. Chlorophyll Content and Carotenoid Content

Salt-alkalization and maize hybrids recorded significant differences in chlorophyll and carotenoid contents in maize leaves (Figure 3). Under salinity stress, Chl a, Chl b, total chlorophyll, and carotenoid contents in both hybrids were reduced by 6–33% compared with the control. Exposure of alkalinity stress also reduced the Chl a content by 17% and 19%, Chl b content by 11% and 18%, total chlorophyll content by 12% and 18%, and carotenoid content by 48% and 53% in DK-6714 and FH-1231, respectively, as compared with control treatments. The negative effects of alkalinity stress were more severe than salinity stress. Compared with FH-1231, DK-6714 recorded less reduction in the accumulation of photosynthetic pigments under stress conditions (Figure 3).

Figure 3. Effect of salinity and alkalinity stress on (A) chlorophyll a, (B) chlorophyll b, (C) chlorophyll a + b, and (D) carotenoid content of 20-day-old maize seedlings of two hybrids (DK-6714 and FH-1231). The bars represent the standard error (SE) of the four replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

3.4. Ions Accumulation

Salt treatments and maize hybrids significantly affected ion accumulation (Figure 4). Under salinity stress, K^+ ion accumulation in both hybrids was significantly decreased (8–25%) compared with control. However, salinity stress increased the Na^+ ions in DK-6714 and FH-1231 by 8% and 12%, respectively, over control. Exposure to alkalinity stress reduced leaf K^+ concentration by 20% and 25% while increasing leaf Na^+ concentrations by 23% and 25% in DK-6714 and FH-1231, respectively, compared with control. The hybrid DK-6714 was comparatively better than FH-1231 with regard to the maintenance of ionic balance under stress conditions (Figure 4).

Figure 4. Effect of salinity and alkalinity stress on (A) Na⁺ ions and (B) K⁺ ions in 20-day-old maize seedlings of two hybrids (DK-6714 and FH-1231). The bars represent the standard error (SE) of the four replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

3.5. Hydrogen Peroxide and Lipid Peroxidation

Compared with the control, salinity stress increased the H₂O₂ content in DK-6714 and FH-1231 by 18% and 19% and the MDA content by 36% and 41%, respectively (Figure 5). Similarly, alkalinity stress increased the H₂O₂ content by 36% and 76% and the MDA content by 65% and 72% in DK-6714 and FH-1231, respectively, compared to control. FH-1231 recorded higher H₂O₂ and MDA content than DK-6714 under both salinity and alkalinity stresses (Figure 5).

Figure 5. Effect of salinity and alkalinity stress on (A) hydrogen peroxide (H₂O₂) and (B) malondialdehyde (MDA) content in 20-day-old maize seedlings of two hybrids (DK-6714 and FH-1231). The bars represent the standard error (SE) of the four replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

3.6. Antioxidant Enzymatic Activity

Salt treatments and hybrids significantly affected the activities of antioxidant enzymes (Figure 6). Salinity stress enhanced SOD activity by 25% and 21%, CAT activity by 57% and 54%, POD activity by 19% and 16%, and APX activity by 49% and 47% in DK-6714 and FH-1231, respectively, compared with control. In DK-6714 and FH-1231, alkalinity stress increased SOD activity by 43% and 40%, CAT activity by 81% and 72%, POD activity by 30%

and 29%, and APX activity by 82% and 78%, respectively, compared with control. Under both saline and alkaline stress conditions, the hybrid DK-6714 showed higher activities of antioxidant enzymes compared with the FH-1231 hybrid (Figure 6).

Figure 6. Effect of salinity and alkalinity stress on (A) superoxide dismutase (SOD), (B) peroxidases (POD), (C) catalase (CAT), and (D) ascorbate peroxidase (APX) activities in 20-day-old maize seedlings of two hybrids (DK-6714 and FH-1231). The bars represent the standard error (SE) of the four replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

3.7. Gene Expression Analysis

Both salinity and alkalinity stress significantly induced the expression levels of antioxidant genes (Figure 7). Salinity stress induced the expression levels of the CAT gene by 85% and 43%, the SOD gene by 65% and 38%, the POD gene by 74% and 65%, and the APX gene by 43% and 27% in DK-6714 and FH-1231, respectively, compared to control. On the other hand, alkalinity stress enhanced the expression levels of the CAT gene by 94% and 67%, the SOD gene by 88% and 52%, the POD gene by 97% and 73%, and the APX gene by 61% and 72% in DK-6714 and FH-1231, respectively, compared to control. Under saline and alkaline stress conditions, the hybrid DK-6714 showed a higher expression of antioxidant genes than FH-1231 (Figure 7).

Figure 7. Effect of salinity and alkalinity stress on the expression levels of (A) the *catalase* (CAT) gene, (B) the *superoxide dismutase* (SOD) gene, (C) the *peroxidase* (POD) gene, and (D) the *ascorbate peroxidase* (APX) gene in 20-day-old maize seedlings of two hybrids (DK-6714 and FH-1231). The bars represent the standard error (SE) of three replicates. Similar small letters above bars represent nonsignificant (ns) differences among treatments ($p \leq 0.05$).

4. Discussion

Maize crops are sensitive to high concentrations of neutral and alkaline salts. Increasing crop yields in saline- and alkaline-affected areas is an important task because the increasing amount of salt-alkali soils has reduced the productivity of agricultural land and brought about an ecological crisis to mankind [20]. The present study examined the morphological, physiological, biochemical, and molecular responses of two maize hybrids to salinity and alkalinity stresses and demonstrated the comparative effects of both stresses on morphological processes and the oxidative metabolism of maize hybrids.

In this study, both salinity and alkalinity caused a marked reduction in fresh and dry weights of root and shoot, leaf area, and number of leaves in maize. Although both stresses reduced plant growth and biomass, the impact of alkalinity stress was more severe than salinity stress (Figures 1 and 2). The reduced biomass and growth under saline salts might be due to specific ion toxicity and osmotic imbalance; however, under alkaline salts, the high pH of the soil might also have affected plant growth. High soil pH damages the root cells of rice and inhibits seedling growth, which sometimes led to plant wilting and even death [46–48]. The high concentration of saline and alkaline salts in the rhizosphere inhibits the elongation process of plants by accumulating high Na^+ ions and low K^+ ions into the cells. Potassium ions play an important role in cell osmotic pressure. Due to the greater accumulation of Na^+ ions and the loss of cell expansion pressure, cells become rigid and cannot reach their maximum size [46,47]. Abd-Elgawad et al. [49] observed that under salinity, maize roots accumulated more Na^+ ions and showed more reduction in plant biomass. Under the influence of alkalinity, insufficient nutrient supply caused by high soil pH can inhibit plant growth [50,51]. The present study reveals that salt-alkalization reduced the accumulation of photosynthetic pigments in maize leaves

(Figure 3). Chlorophyll contents (Chl a and b) play a special role in photosynthesis, and a decline in photosynthetic pigments reduces the photosynthesis rate and food supply for plant growth [52,53].

In this study, the chlorophyll contents of both hybrids were decreased under salinity and alkalinity stresses, and such reductions were more severe under alkalinity stress. Several authors have reported that salinity and alkalinity stresses increase the activity of chlorophyll-degrading enzymes, thereby reducing total chlorophyll content [27,54,55]. Alkalinity reduces the uptake of Mg^{2+} ions, which are the building block of chlorophyll and the central atom of the chlorophyll molecule. A decrease in Mg^{2+} concentration under alkaline stress can lead to the degradation of green pigments [18]. Moreover, salt-induced oxidative stress affects the structure of the chloroplast and causes the degradation of chlorophyll in plants. This is due to the increase of chlorophyllase enzyme activity, which leads to a decrease in the photosynthetic rate under alkaline stress [56–58].

For normal function of the enzymatic processes in cells, high K^+ ions and low Na^+ ions in the cytoplasm are essential. In the present study, both salinity and alkalinity stresses increased Na^+ while decreasing the K^+ concentration in maize leaves; however, such effects were prominent under alkalinity stress (Figure 4). The accumulation of Na^+ in cells is the main cause of ionic imbalance because Na^+ is the main toxic inorganic ion in saline conditions [59,60]. With the increase of saline and alkaline toxicity, plants accumulate Na^+ ions, thereby inhibiting the absorption of K^+ ions. Plant species have a Na^+/H^+ antiporter that is involved in the exclusion of Na^+ ions and allow K^+ ions to enter the cell. Nonselective cation channels and high-affinity K^+ transporters play an important role in the excessive accumulation of intracellular Na^+ ions, and Na^+ enters the cell through these transporters and channels [61]. Due to low external proton concentration, salinity and alkalinity reduce the exchange capacity of the Na^+/K^+ antiporters, which leads to a reduction in Na^+ exclusion, which, in turn, results in the higher accumulation of Na^+ ions in the cells [61,62]. Therefore, reducing Na^+ ion exclusion might be the reason for the increase of Na^+ ions in the shoots of both maize hybrids. Under alkalinity stress, osmotic stress is not the cause of the increase of Na^+ ions in the cells, while high pH is the cause of the toxicity of specific ions [28]. The high pH value caused by alkalinity also reduces the ability of roots to absorb Na^+ and K^+ ions and disturbs the balance between Na^+ and K^+ ions [9,63]. Under the influence of alkalinity, the disturbance of ionic balance sharply reduces the K^+ ions in plant shoots [28].

The excessive accumulation of ROS caused by salinity and alkalinity enhances oxidative damage in plants. Reactive oxygen species such as H_2O_2 have a significant role in injuries caused by salt and alkali stresses. In this study, salt-alkalization significantly increased the H_2O_2 content in both maize hybrids, but this increase was greater under alkaline stress (Figure 5). Soil alkalinity causes acid-base disturbance in cells, and H_2O_2 is produced due to high pH [34].

Membrane damage is measured by MDA content, which is the decomposed product of polyunsaturated fatty acid hydroperoxides. As a result of lipid peroxidation, MDA content accumulates in the cells. Compared with tolerant varieties, sensitive plants accumulate more MDA content; therefore, it is used to measure plant tolerance [64,65]. In this study, an increase in MDA content was observed in both maize hybrids, but hybrid FH-1231 accumulated more MDA content than DK-6714 (Figure 5). Membrane damage is the primary effect of salt stress [66]. The injurious effects of neutral and alkaline salts on the membrane structure increase with the increase of salt concentration [20]. Specific ion toxicity leads to the accumulation of high MDA content due to membrane damage. During alkaline stress, excess Na^+ ions participate in the production of ROS by acting as signal molecules in the signal transduction pathway. Excessive production of ROS under salt stress can cause cell toxicity and cell damage, leading to cell death [56].

In order to maintain the balance between cell redox and detoxification of ROS, several enzymatic and nonenzymatic antioxidants play key roles under abiotic stresses. Tolerant varieties produce large amounts of antioxidants to deal with oxidative stress [67]. The

results of the present study show that salinity and alkalinity conditions significantly regulated the activities of SOD, CAT, POD, and APX, as well as the expression levels of different antioxidant genes (Figures 6 and 7). Superoxide dismutase is an enzyme that decomposes superoxide radicals (O_2^-) into H_2O_2 . In order to overcome ROS, SOD is at the front line. They are classified according to their position in the cell and metal cofactors (such as Cu/Zn, Fe, Mn, and Ni). In plant development, environmental and tissue-specific signals are involved in the regulation of SOD [68–70]. After SOD dismutation of O_2^- to H_2O_2 , further dismutation of H_2O_2 takes place by CAT, and CAT is abundantly localized in peroxisomes. It has been found that salt stress increases CAT levels in pearl millet at reproductive and vegetative stages [71]. POD decomposition of H_2O_2 in chloroplasts is produced by SOD dismutation of O_2^- [72]. APX is present in mitochondria, chloroplast, cytosol, and peroxisomes. APX is a member of Class-I heme peroxidase and exists in the protist kingdom [73,74], red algae [75], and higher plants [76,77]. By using ascorbate as an electron donor, it participates in the catalysis of H_2O_2 to H_2O [78,79]. El-Esawi et al. [80] indicated that salinity stress induced the expression levels of antioxidant genes (APX, CAT, SOD) in maize plants compared to control. Furthermore, Lin and Pu [81] revealed that salt-tolerant varieties have higher APX levels than salt-sensitive ones, which are in agreement with the results recorded in the current study.

5. Conclusions

Both salinity and alkalinity stresses reduced the biomass and growth of both maize hybrids, disrupted the ionic balance, increased the lipid peroxidation rate (MDA content) and H_2O_2 concentration, enhanced the activities of antioxidant enzymes (SOD, CAT, POD, and APX), and triggered the expression levels of antioxidant genes. For all studied traits, the negative effects of alkalinity stress were more severe than salinity. Under saline and alkaline stress conditions, DK-6714 performed better than FH-1231 and showed less reduction in growth and biomass. The greater performance of DK-6714 was associated with the higher activity of antioxidant enzymes, the maintenance of photosynthetic pigments, and the ionic balance under stress conditions.

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