Jasmonic acid ameliorates alkaline stress by improving growth performance, ascorbate glutathione cycle and glyoxylase system in maize seedlings

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Environmental pollution by alkaline salts, such as Na\(_2\)CO\(_3\), is a permanent problem in agriculture. Here, we examined the putative role of jasmonic acid (JA) in improving Na\(_2\)CO\(_3\)-stress tolerance in maize seedlings. Pretreatment of maize seedlings with JA was found to significantly mitigate the toxic effects of excessive Na\(_2\)CO\(_3\) on photosynthesis- and plant growth-related parameters. The JA-induced improved tolerance could be attributed to decreased Na uptake and Na\(_2\)CO\(_3\)-induced oxidative damage by lowering the accumulation of reactive oxygen species and malondialdehyde. JA counteracted the salt-induced increase in proline and glutathione content, and significantly improved ascorbic acid content and redox status. The major antioxidant enzyme activities were largely stimulated by JA pretreatment in maize plants exposed to excessive alkaline salts. Additionally, increased activities of glyoxalases I and II were correlated with reduced levels of methylglyoxal in JA-pretreated alkaline-stressed maize plants. These results indicated that modifying the endogenous Na\(^{+}\) and K\(^{+}\) contents by JA pretreatment improved alkaline tolerance in maize plants by inhibiting Na uptake and regulating the antioxidant and glyoxalase systems, thereby demonstrating the important role of JA in mitigating heavy metal toxicity. Our findings may be useful in the development of alkali stress tolerant crops by genetic engineering of JA biosynthesis.

Agricultural soil contamination by alkaline salts has been recognized for the past few decades; however, exposure to alkalinity still continues and is worsening, principally in Asian countries1. In this context, alkaline stress is among the most crucial environmental constraints in arid and semi-arid environments, affecting agricultural crop productivity globally2. Excessive alkaline stress can induce numerous negative effects in plants at the cellular level by accumulating high Na and promoting ionic stress, osmotic stress by inducing water deficit, and ultimately resulting in the overproduction of reactive oxygen species (ROS) and oxidative stress3–5. Higher levels of alkaline salts in plant growth medium reduce K\(^{+}\) content and enhance Na uptake and accumulation, causing an efflux of K\(^{+}\) ions and promoting K\(^{+}\) leakage from plant cells6–8. Moreover, under alkaline stress conditions, Na content rises above that of K\(^{+}\), resulting in poor nutrient uptake and lack of Na/K homeostasis8. A slightly higher pH than the ideal level of alkaline salts is toxic and can adversely affect physio-biochemical processes, including mineral uptake, photosynthesis, membrane integrity, and yield of plants6–8. Higher pH also causes abnormality in root morphology, causing leaf chlorosis and necrotic lesions in leaves, all of which hamper plant growth and development, and ultimately lead to reduced crop yield9.

The inborn redox nature of alkaline salts, such as Na\(_2\)CO\(_3\), also accelerates toxicity by generating ROS, such as superoxide anions (O\(_{2}^{-}\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxyl radicals (OH\(^{−}\))9,10. To overcome

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alkaline-induced oxidative stress, plant cells are well furnished with inherent antioxidant capability that is comprised of enzymatic components, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX), glutathione S-transferase (GST), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR), as well as non-enzymatic components, such as glutathione (GSH) and ascorbic acid (AsA)\textsuperscript{11,12}. Being a highly reactive compound, methylglyoxal (MG) accumulation is well known to occur under various abiotic stresses, including alkaline-salt stress\textsuperscript{13}. Moreover, salt stress increases its cytotoxicity on biomolecules, and causes brutal oxidative damage to proteins either (i) directly through accelerating ROS production, or (ii) indirectly by the overproduction of advanced glycation end products (AGEs)\textsuperscript{14,15}. To overcome MG toxicity, plants normally contain a GSH-dependent glyoxalase (Gly) system that converts MG into D-lactate by employing Gly I and Gly II\textsuperscript{14}. Effective performance of antioxidant defence and the Gly systems corroborates enhanced tolerance to various abiotic stresses\textsuperscript{13}. Maintaining Na and K homeostasis is critical in regulating the intracellular Na content to prevent toxicity. Plants normally have numerous mechanisms that hamper alkaline toxicity, such as inhibition of Na uptake, and prevention of its accumulation by binding with exudates of roots, intracellular sequestration by phytochelatins and strong ligands, such as cysteine-rich compounds\textsuperscript{9}.

Maize (Zea mays L.) is an important cereal food crop grown worldwide and is also considered a high fibre yielding crop in many Asian countries, including China, India and Pakistan. India is the world’s largest producer of maize, with 8.4 million hectares under cultivation\textsuperscript{16}. Cultivation of cereal crops, such as maize, in alkaline salt polluted lands, results in reduced yield and seed quality. In India, agricultural lands are exceedingly polluted with alkaline salts because of unrestrained water shortages and climate change\textsuperscript{17}. Therefore, the development of maize varieties tolerant to alkaline stress or the elucidation of the entire mechanism of maize plant responses to alkaline toxicity is essential for sustainable maize production.

Plant responses to environmental stimuli are mostly orchestrated by an array of plant growth regulators, including phytohormones. Jasmonic acid (JA) and methyl jasmonate (MeJA) have been known to activate a number of signalling events during plant responses to abiotic and biotic stresses, thus developing improved safeguards in plants under these stresses\textsuperscript{18}. Modification of endogenous JA levels in plants appeared to be a promising method of providing protection against numerous abiotic stresses, including salinity, heat, drought, and metal toxicity\textsuperscript{18,19}. However, knowledge of the interactions of JA during alkaline stress and how JA modulates the physiological and biochemical changes under alkaline stress in an economically important cereal crop, such as maize, remains elusive. Moreover, available information regarding the precise roles of JA in its simultaneous regulation of ROS and detoxification of MG under alkaline stress in crop plants is limited, and thus warrants in-depth investigation to understand how antioxidant metabolism changes in crop plants in response to Na\textsubscript{2}CO\textsubscript{3} induced-toxicity. In the current study, our goal was to investigate the effects of JA on growth and physio-biochemical processes in maize by considering the mechanisms related to (i) Na\textsuperscript{+} and K\textsuperscript{+} uptake and homeostasis, (ii) JA-induced alterations in growth performance and oxidative parameters, (iii) the role of JA in the modification of non-enzymatic and enzymatic defences, and (iv) MG detoxification under alkaline-stress conditions. The current study was undertaken to evaluate the effects of JA on growth, ion homeostasis, antioxidants, and methylglyoxal metabolizing enzyme activity in the amelioration of alkaline stress in maize plants.

**Results**

**JA improves maize plant phenotypes.** As depicted in Fig. 1, alkaline (Na\textsubscript{2}CO\textsubscript{3}) treatment induced yellowing symptoms on maize leaves. Alkaline treatment also hampered plant growth in terms of plant height and leaf length. Exogenous JA priming mitigated the negative effects of Na\textsubscript{2}CO\textsubscript{3} and improved phenotypes of maize seedlings.

**Pretreatment with JA improves growth.** Seedlings fed only Na\textsubscript{2}CO\textsubscript{3} showed reduced shoot height by 46.07 and 66.33\% and reduced leaf length by 45.02 and 76.36\% at 100 mM and 150 mM Na\textsubscript{2}CO\textsubscript{3}, respectively, as compared to that of the control (0 mM Na\textsubscript{2}CO\textsubscript{3} + 0\µM JA). However, priming application of JA to alkaline treated plants relieved the toxic effects of Na\textsubscript{2}CO\textsubscript{3} and enhanced the shoot height and leaf length of seedlings (Fig. 2A,B). Root length was reduced by 63.62 and 78.01\% in alkaline treated plants in the 100 mM and 150 mM Na\textsubscript{2}CO\textsubscript{3} treatments, respectively, in comparison with that of the untreated control (Fig. 2A). Pretreatment of JA enhanced...
root length by 16.91% at 100 mM Na₂CO₃ + 10 µM JA and 12.37% at 150 mM Na₂CO₃ + 10 µM JA relative to the 100 and 150 mM Na₂CO₃ treatments, respectively. Seedling biomass in terms of fresh and dry weights (FW and DW) indicated dramatic declines under alkaline stress (Fig. 2C). Seedling DW under Na₂CO₃ stress alone was decreased by 50.00% at 100 mM Na₂CO₃ whereas at 150 mM Na₂CO₃ the maximum reduction in dry weight 90.90% was recorded (Fig. 2C). However, supplementation of JA to Na₂CO₃ fed seedlings resulted in improved DW, which demonstrated the positive effects of JA on alkaline stress.

Seed priming with JA maintains RWC, chlorophyll pigments, soluble proteins, soluble sugars, and proline content. RWC under Na₂CO₃ stress significantly declined by 21.99% at 100 mM Na₂CO₃, but the maximum decline of 31.65% in RWC was recorded at 150 mM Na₂CO₃ compared with that of the untreated control (Fig. 2D). However, a notable increase in RWC (17.96 and 13.39%) was recorded for the 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA treatments, respectively, in the JA-primed alkaline-stressed seedlings compared with those fed only 100 mM Na₂CO₃ and 150 mM Na₂CO₃.

Data presented in Table 1, show that alkaline stress reduced the biosynthesis of photosynthetic pigments in maize leaves. The maximum reduction in total Chl and carotenoids of 67.79 and 60.00%, respectively, was recorded in the 150 mM Na₂CO₃ treatment compared with that of the control untreated plants. Pretreatment with JA for alkaline stressed plants mitigated the toxic effects of Na₂CO₃ and improved the Chl content by 41.26 and 32.43% and carotenoids by 40.11% and 24.00% in 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA treatments, respectively, in the JA-primed alkaline-stressed seedlings compared with those fed only 100 mM Na₂CO₃ and 150 mM Na₂CO₃.

The concentration of the osmoprotective proline significantly increased by 23.12% and 153.74% in 100 mM Na₂CO₃ and 150 mM Na₂CO₃ alkaline-stressed seedlings relative to the control (Table 1). Supplementation of JA to Na₂CO₃-treated seedlings showed a steep decline in proline content by 23.12% and 131.67%, respectively, at 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA as compared with only alkaline treated 100 and 150 mM Na₂CO₃ seedlings. However, proline content declined by 10.52% with supplementation of JA over the entire investigational period in non-stressed (10 µM JA + 0 mM Na₂CO₃) seedlings.

The Na₂CO₃ induced stress caused a significant reduction in total soluble sugars (TSSs) by 15.09 and 27.32% in 100 mM Na₂CO₃ and 150 mM Na₂CO₃ treatments, respectively, for maize seedlings. Application of JA mitigated the adverse effects of alkaline stress and caused significant TSSs accumulation of 2.22 and 12.73% in 100 mM
Na$_2$CO$_3$ + 10µM JA and 150 mM Na$_2$CO$_3$ + 10µM JA treatments, respectively, compared to only alkaline stressed plants with 100 and 150 mM 10µM JA + 0 mM Na$_2$CO$_3$, respectively (Table 1).

In the alkaline stressed plants, the soluble protein content was dramatically reduced by 17.64 and 28.87% in 100 and 150 mM Na$_2$CO$_3$ treatments, respectively, in comparison with that of 0 mM Na$_2$CO$_3$ + 0 µM JA untreated control plants. Applying JA to alkaline stressed plants alleviated the adverse effects of Na$_2$CO$_3$ and enhanced the total soluble protein content by 11.49 and 9.14% in the 10µM JA + 100 mM Na$_2$CO$_3$ and 10µM JA + 150 mM Na$_2$CO$_3$ treatments, respectively. Moreover, application of JA to unstressed maize plants increased protein content by 14.66% in 10µM JA + 0 mM Na$_2$CO$_3$ treatment in comparison with that of the non-treated control (Table 1).

### JA attenuates Na$^+$ toxicity, modulates Na$^+$ and K$^+$ homeostasis.

In this study, exogenous JA caused a decline in Na$^+$ uptake in roots, as well as in leaves of maize seedlings. The roots of only Na$_2$CO$_3$ fed seedlings exhibited enhancement in Na$^+$ content by 6.5-fold in 100 mM Na$_2$CO$_3$ and 10.5-fold in 150 mM Na$_2$CO$_3$ treatments, as compared with that of the control (Fig. 3A). The uptake of Na$^+$ declined by 21.88 and 52.80% in JA supplemented alkaline-fed 100 mM Na$_2$CO$_3$ + 10µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA seedlings as compared to only 100 and 150 mM Na$_2$CO$_3$ treated seedlings, respectively (Fig. 3A).

Similarly, the leaves of 100 and 150 mM alkaline fed seedlings showed a 6.5-fold and 10.5-fold increase in Na$^+$ content in comparison with that of the control group plants. However, pretreatment of JA to alkaline stressed seedlings resulted in a 21.94% and 52.69% reduction in Na$^+$ content in 100 mM Na$_2$CO$_3$ + 10µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA treatments, respectively, as compared to only 100 and 150 mM alkaline stressed seedlings (Fig. 3A). Furthermore, the JA primed seedlings showed decreased uptake of Na$^+$ in leaves by 12.30% compared with that of the control seedlings.

Conversely, the reverse trend was noticed for K$^+$ content in roots and the reduction in K$^+$ content was 1.7 and 2.8-folds in 100 and 150 mM Na$_2$CO$_3$, respectively, as compared to that of the untreated control plants (Fig. 3B). Supplementation with JA to 100 and 150 mM alkaline fed seedlings showed enhanced root K$^+$ content by 43.51 and 78.49% as compared to only 100 and 150 mM Na$_2$CO$_3$ stressed plants. Likewise, a steep decline in shoot K$^+$ content by 59.68 and 113.57% was recorded in the 100 and 150 mM alkaline fed maize plants. However, exogenous supplementation of JA to alkaline stressed seedlings exhibited a 21.94 and 52.07% increase in shoot K$^+$ in 100 mM Na$_2$CO$_3$ + 10 µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA as compared to that of only 100 and 150 mM Na$_2$CO$_3$ fed plants (Fig. 3B). Additionally, the Na/K ratio in both roots and shoot was higher in the only 100 and 150 mM alkaline fed maize seedlings in comparison to that of the untreated control plants. Supplementation of JA to alkaline fed seedlings resulted in a significant reduction in the Na/K ratio in roots and leaves. The present data revealed that JA priming attenuates Na$^+$ toxicity and protects seedlings from injuries (Fig. 3C).

### Effects of JA on H$_2$O$_2$ contents and malondialdehyde (MDA).

The production of H$_2$O$_2$ was greatly increased by 96.85% with 100 mM Na$_2$CO$_3$, which furthermore was enhanced to 154.16% in the 150 mM Na$_2$CO$_3$ treatment as compared to that of the control untreated plants. Suppling JA to the Na$_2$CO$_3$ treated plants resulted in reductions in H$_2$O$_2$ production by 51.81 and 46.41% at 10µM JA + 100 mM Na$_2$CO$_3$ and 10µM JA + 150 mM Na$_2$CO$_3$, respectively, in comparison with only 100 and 150 mM Na$_2$CO$_3$ alkaline fed plants (Fig. 4A). In addition, under unstressed conditions, exogenous JA evinced a 0.69% increase in the contents of H$_2$O$_2$ for that of 0 mM JA + 0 mM Na$_2$CO$_3$, to ensure the protective signalling role of JA. Compared with untreated control seedlings, MDA content was increased by 63.45% in 100 mM Na$_2$CO$_3$ and 92.26% in 150 mM Na$_2$CO$_3$ stressed plants (Fig. 4A). However, application of JA to Na$_2$CO$_3$ fed plants showed a 17.29 and 21.36% reduction in lipid peroxidation at 100 mM Na$_2$CO$_3$ + 10µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA, respectively, as compared to only alkaline-stressed plants at 100 and 150 mM Na$_2$CO$_3$, respectively.

### Effects of JA on lipoxygenase (LOX) activity and (%) electrolyte leakage (EL).

Lipoxygenase (LOX) activity was amplified by 99.44% in 100 mM Na$_2$CO$_3$ and 167.01% in 150 mM Na$_2$CO$_3$ fed maize plants, compared with that of the control (Fig. 4B). However, priming treatment of JA to alkaline fed plants showed a decrease in LOX activity by 77.94% at 10µM JA + 100 mM Na$_2$CO$_3$ and 74.04% at 10µM JA + 150 mM Na$_2$CO$_3$, compared with the 100 and 150 mM Na$_2$CO$_3$ treatments. In non-stressed conditions (10µM JA + 0 mM Na$_2$CO$_3$), JA slightly altered LOX activity in seedlings relative to that of the control. The Na$_2$CO$_3$ treated plants exhibited an 81.10 and 130.47% increase in % EL as compared to that of the control seedlings at 100 and 150 mM Na$_2$CO$_3$.
respectively, but pretreatment of JA to alkaline stressed plants showed a 60.18 and 54.52% reduction in EL at 100 mM Na$_2$CO$_3$ + 10 µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA, respectively, as compared to only Na$_2$CO$_3$ fed plants (Fig. 4C).

JA maintains ascorbic acid (AsA) content, DHA, and AsA/DHA ratio. In comparison with that of the control (0 mM Na$_2$CO$_3$ + 0 µM JA) untreated plants, the level of total AsA declined significantly for both 100 and 150 mM Na$_2$CO$_3$-fed seedlings, with a steep decrease of 16.54 and 29.81% recorded for the 100 and 150 mM Na$_2$CO$_3$ treatments, respectively (Table 2). However, Na$_2$CO$_3$ stressed plants supplemented with JA showed enhancement in AsA concentrations by 9.94 and 3.54% more in 10 µM JA + 100 mM Na$_2$CO$_3$ and 10 µM JA + 150 mM Na$_2$CO$_3$ treatments, respectively, in comparison with alkaline only stressed plants 100 and 150 mM Na$_2$CO$_3$ respectively. The concentration of dehydroascorbic acid (DHA) significantly decreased by 40.55 and 35.11% in the 100 mM Na$_2$CO$_3$ and 150 mM Na$_2$CO$_3$ treatments, respectively, in alkaline stressed maize plants. However, JA pretreatment of Na$_2$CO$_3$ stressed plants resulted in the reduction in DHA concentration by 23.00 and 26.74% in the 100 mM Na$_2$CO$_3$ + 10 µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA treatments, respectively, as compared to alkaline only treated 100 and 150 mM Na$_2$CO$_3$ plants. The AsA/DHA ratio declined considerably in the alkaline-stressed seedlings at both 100 and 150 mM treatment levels, as compared to that of the control, and dropped severely by 63.86% at 100 mM Na$_2$CO$_3$ and 76.07% at 150 mM Na$_2$CO$_3$; however, the ratios of AsA/DHA in the JA-primed alkaline-fed 10 µM JA + 100 mM Na$_2$CO$_3$ and 10 µM JA + 150 mM Na$_2$CO$_3$ seedlings was 35.40 and 31.37% higher compared with the only Na$_2$CO$_3$-treated seedlings at 100 and 150 mM Na$_2$CO$_3$ treatments, respectively, suggesting stress resistance (Table 2).

Effects of JA on GSH content and GSH to oxidized GSH (GSSG) ratio (GSH/GSSG). Maize seedlings stressed with Na$_2$CO$_3$ increased their GSH content by 11.88% with only 100 mM Na$_2$CO$_3$, compared with that of the control (Table 2). Applying JA to alkaline-stressed plants efficiently enhanced the GSH level up to 11.34 and 26.20% with 10 µM JA + 100 mM Na$_2$CO$_3$ and 10 µM JA + 150 mM Na$_2$CO$_3$ treatments, respectively, compared with only 100 and 150 mM alkaline-stressed plants. However, application of JA to non-stressed plants (10 µM JA + 0 mM Na$_2$CO$_3$) resulted in 13.12% higher levels of GSH contents as compared to that of the control. Alkaline stressed maize plants showed a dramatic decline in GSSG content by 29.92 and 54.72% with the 100 and 150 mM Na$_2$CO$_3$ treatments, respectively, when compared to that of the untreated control plants. Addition of
Figure 4. Effects of jasmonic acid (JA) on the (A) H$_2$O$_2$ and MDA content, (B) LOX and (C) EL in maize plants with and without alkaline stress. Bars represent standard deviation (SD) of the mean ($n=3$). Different letters (a, b, c, d and e) indicate statistically significant differences among the treatments, according to Duncan's multiple range test at ($P<0.05$). FW, fresh weight.

| Treatment | AsA (nmol g$^{-1}$ FW) | DHA (nmol g$^{-1}$ FW) | AsA/DHA ratio | GSH (nmol g$^{-1}$ FW) | GSSG (nmol g$^{-1}$ FW) | GSH/GSSG ratio |
|-----------|------------------------|------------------------|---------------|------------------------|------------------------|-----------------|
| Control   | 210.52 ± 5.17$^b$      | 46.90 ± 4.12$^a$       | 4.49 ± 0.62$^a$ | 159.25 ± 6.17$^d$    | 12.50 ± 1.07$^c$     | 12.82 ± 2.10$^a$ |
| 100 mM    | 180.64 ± 6.22$^a$      | 65.92 ± 6.08$^b$       | 2.74 ± 0.42$^d$ | 178.17 ± 6.22$^c$    | 16.24 ± 2.07$^b$    | 10.98 ± 1.19$^a$ |
| 150 mM    | 162.07 ± 4.35$^c$      | 54.37 ± 2.07$^b$       | 2.98 ± 0.23$^d$ | 168.74 ± 4.15$^d$    | 19.34 ± 3.05$^c$    | 8.73 ± 1.51$^d$  |
| C+JA      | 214.79 ± 6.34$^a$      | 46.59 ± 5.10$^a$       | 4.61 ± 0.51$^a$ | 160.14 ± 3.34$^c$    | 14.34 ± 1.04$^c$    | 11.18 ± 2.62$^a$ |
| 100 mM+JA | 198.60 ± 8.24$^c$      | 53.59 ± 5.11$^b$       | 3.71 ± 0.44$^d$ | 198.38 ± 6.24$^c$    | 19.01 ± 2.14$^a$    | 10.49 ± 1.68$^a$ |
| 150 mM+JA | 167.81 ± 5.78$^a$      | 50.01 ± 4.12$^b$       | 3.35 ± 0.21$^a$ | 187.72 ± 4.65$^d$    | 19.92 ± 2.45$^d$    | 9.44 ± 3.78$^e$  |

Table 2. Effects of jasmonic acid (JA) on the contents of non-enzymatic antioxidants, ascorbic acid (AsA), dehydroascorbate (DHA), AsA/DHA ratio, reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG ratio in maize plants with or without alkaline stress. Values are means ± SD of three independent replications ($n=3$). Different letters (a–f) within the column indicate statistically significant differences among the treatments, according to Duncan's multiple range test at ($P<0.05$). FW, fresh weight.
JA to alkaline stressed plants resulted in 17.055 and 3.015% increase in GSSG concentration in comparison with that of only 100 and 150 mM Na₂CO₃ stressed plants, respectively. The alkaline only fed plants showed a 16.75 and 46.48% decrease in the GSH/GSSG ratio at 100 and 150 mM Na₂CO₃ treatment levels compared with that of the (0 mM Na₂CO₃ + 0 µM JA) control. However, application of JA to Na₂CO₃-stressed plants resulted in higher GSH/GSSG ratios than those of the only 100 and 150 mM Na₂CO₃-stressed seedlings at both 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA treatment levels (Table 2).

**Effects of JA on Antioxidant enzymes SOD, CAT, GPX, and GST.** The slight increase in superoxide dismutase activity 7.26% was recorded at 100 mM Na₂CO₃ level of treatment relative to that of the untreated control. JA-priming of alkaline stressed seedlings of maize was able to enhance SOD activity (9.82 and 8.59%) at both the 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA treatments, respectively, compared with only alkaline treatments of 100 mM and 150 mM, suggesting a complex effect of JA on modulation of SOD activity (Fig. 5).

The maize seedlings exposed to alkaline stress had increased CAT activity by 28.85 and 16.53% at 100 and 150 mM, respectively, as compared with that of the non-treated control (Fig. 5). In contrast, JA-supplementation to alkaline-stressed seedlings showed enhanced CAT activity by 5.40% at 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA, as compared to only 100 and 150 mM Na₂CO₃ stressed seedlings, respectively. Moreover, in comparison with that of the control (0 mM Na₂CO₃ + 0 µM JA) JA-priming to unstrained plants elevated catalase activity by 8.33% with the (0 mM Na₂CO₃ + 10 µM JA) treatment. The maize seedlings exposed to alkaline stress had reduced GPX activity by 5.26% at the 100 mM Na₂CO₃ and 17.56% at the 150 mM Na₂CO₃ level compared with control (Fig. 5). Exogenous JA to alkaline-fed plants had increased GPX activity by 14.06 and 15.85% at 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA levels compared with only seedlings treated with 100 and 150 mM Na₂CO₃, respectively. In non-stressed plants, application of JA enhanced GPX activity by 34.46% at (0 mM Na₂CO₃ + 10 µM JA) treatments in comparison with that of the control plants. Seedlings exposed to Na₂CO₃ had decreased GST activity by 27.35 and 59.70% at 100 and 150 mM levels, respectively, compared with that of the control untreated plants (Fig. 5). In contrast, JA-priming to Na₂CO₃ stressed maize plants had 78.94 and 110.71% increased GST activity in 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM J treatments in comparison with only that of the 100 and 150 mM alkaline-stressed plants, respectively.

**Activities of ascorbate-glutathione cycle enzymes.** The results related to the activities of the ascorbate-glutathione cycle enzymes are depicted in Fig. 6. Under alkaline stress, APX activity increased by 19.89 and 2.10% at 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA levels, respectively, compared with that of the control (Fig. 6). APX activity significantly increased by 17.29 and 21.36%, respectively, at 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA levels in the JA-pretreated alkaline-stressed seedlings compared with the 100 and 150 mM alkaline-stressed only seedlings, respectively. APX activity increased significantly in the JA-primed seedlings at (0 mM Na₂CO₃ + 10 µM JA) level only, relative to that of the control. MDHAR activity increased by 74.27 and 49.05% in 100 and 150 mM alkaline stressed seedlings compared with that of the control (Fig. 6). JA-priming of Na₂CO₃ stressed plants showed enhanced MDHAR activity by 19.34 and 27.33% in 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA treatments, respectively, in comparison with that of 100 and 150 mM Na₂CO₃ treatments. DHAR activity increased by 16.19 and 18.74% in 100 and 150 mM alkaline-stressed seedlings when compared with the control, respectively (Fig. 6). On the other hand, DHAR activity increased by 26 and 27% in the JA-pretreated Na₂CO₃ stressed seedlings compared with the alkaline-stressed only (100 mM Na₂CO₃) and (150 mM Na₂CO₃) treated seedlings, respectively. A significant change in DHAR activity was observed in the maize seedlings under non-stressed conditions upon JA pretreatment over the experimental period. GR activity decreased by 20.47 and 14.45% at 100 and 150 mM Na₂CO₃ stressed seedlings relative to that of the control respectively (Fig. 6). In addition, GR activity increased by 14.24 and 14.49% in the JA-pre-treated alkaline-stressed 100 mM Na₂CO₃ + 10 µM JA and 150 mM Na₂CO₃ + 10 µM JA seedlings compared with the 100 and 150 mM only alkaline fed seedlings.
Modulation of glyoxalase system. Seedlings under alkaline stress linearly enhanced the concentration of MG by 27.75% at (100 mM Na₂CO₃) level and 56.71% at (150 mM Na₂CO₃) level compared with that of the control seedlings (Fig. 7A). However, application of JA to Na₂CO₃ stressed plants showed reduction in the levels of MG concentration by 32.48% and 27.98% at both (100 mM Na₂CO₃ + 10 µM JA) and (150 mM Na₂CO₃ + 10 µM JA) levels respectively, and declined the Na₂CO₃-induced toxicity. The activity of enzyme Gly I reduced by 12.74% at 100 mM and 34.17% at 150 mM alkaline stressed plants in comparison to control (0 mM Na₂CO₃ + 0 µM JA) plants (Fig. 7B). JA supplementation considerably amplified Gly II activity by 38.27 and 33.34% with...
100 mM Na$_2$CO$_3$ + 10 µM JA and 150 mM Na$_2$CO$_3$ + 10 µM JA treatments, compared with the 100 and 150 mM Na$_2$CO$_3$- only fed seedlings, respectively. However, a significant rise in the activity of Gly II was evinced under non-stressed conditions upon JA-priming over control plants.

**Discussion**

In this study, we have provided information regarding how JA regulates ion homeostasis to confer a shield on maize plants against high alkaline salts. Alkaline stress has been shown to hamper overall plant growth of maize plants (Fig. 1). In the current study, a dramatic decline was recorded in plant height, root length, leaf length, FW, and DW of maize seedlings fed with 150 mM alkaline salt, perhaps by accumulation of high Na content, caused osmotic stress by impaired ion homeostasis and hampered overall growth performance. However, pretreatment of JA to salt affected seedlings restored the plant growth and biomass. These results are in agreement with the findings of Keramat et al.$^{20,21}$ in which exogenous JA counter abiotic stress constraints and restored growth. Alkaline stress dramatically reduced leaf relative water content (LRWC), and this might have been caused by osmotic stress, which stimulates the accumulation of osmoprotectant proline and induced physiological water deficit conditions and hampers water uptake. The priming treatment of both JA to alkaline stressed seedlings displayed improved RWC and maintains proline accumulation as high as the control group. This result is in consistent with the findings of Poonam et al.$^{22}$ who reported that exogenous JA restored the RWC and Pro accumulation in Cajanus cajan copper-fed seedlings.

Alkalinity drastically declined the leaf chlorophyll pigments in the present study (Table 1). Our results were similar to those Abdel Latef and Tran$^{23}$ who reported that alkaline toxicity reduced chlorophyll contents in *Morus alba* and *Z. mays* plants subjected to alkalinity. Reduction in chlorophyll pigments might have been caused by inefficient activities of the enzymes proto chlorophyllide reductase and α-aminolevulinic acid dehydratase (ALA-dehydratase), which are coordinately involved in biosynthesis of chlorophyll$^{24}$. Moreover, supplementation of JA improved shoot dry weight and chlorophyll content under multiple stress conditions$^{25}$ in wheat$^{25}$ and soybean$^{21}$. Accumulation of compatible osmolyte proline under alkaline stresses has been reported to be a noble indicator of stress tolerance$^{26,27}$. Proline helps in osmotic adjustment, restoration of chlorophyll pigment molecules$^{27}$. Application of JA is reported to enhance the proline content in *Glycine max* under Ni stress$^{21}$. The enzymes related to cause accumulation of mRNAs encoding, proline-rich proteins might be stimulated by JA and protect the cell from the oxidative burst by scavenging ROS$^{38}$.

H$_2$O$_2$ is a very noxious ROS and drastically increased with increasing Na$_2$CO$_3$ concentration and the results of the present study support the findings of Ahmad et al.$^{21}$ in mulberry plants. Under salt stress, a product of membrane peroxidation MDA is frequently used as a prime indicator of oxidative stress$^{38}$. Nahar et al.$^{13}$ observed amplified MDA content because of a salt induced oxidative burst in mung beans. In addition, alkalinity was reported to increase H$_2$O$_2$ and LOX enzyme activity, which prompted lipid peroxidation. Furthermore, Abdel Latef and Tran$^{8}$ also reported the enhancement in MDA content with higher alkaline concentrations in maize. Indeed, JA pre-treatment reduces the creation of H$_2$O$_2$ and other free radicals which openly affect the lipid membranes. The present result is supported by Sirhindi et al.$^{38}$ who found overproduction in MDA content in soybean under Ni stress was significantly reduced by exogenous JA application. Poonam et al.$^{22}$ have confirmed the reduced level of lipid peroxidation in *Cajanus cajan* to JA under copper toxicity. Therefore, pre-treatment of JA showed shielding nature on lipid membrane by decreasing production of H$_2$O$_2$ and superoxide radicles and alleviating lipid peroxidation by increasing the transcript levels and activities of SOD, POD, CAT and APX and the contents of GSH$^{25}$.

The chief enzymatic network that detoxifies ROS is superoxide dismutase, catalase, peroxidases, and ascorbate-glutathione cycle enzymes$^{12}$. The increase in antioxidant activities in the present study corroborates with the findings of Sirhindi et al.$^{21,22}$ SOD is believed to serve as frontline antioxidant defence against various environmental stress regimes, including the salinity and catalysing O$_2^-$ into H$_2$O$_2$, thereafter subsequently removed by CAT and GPX$^{12}$. In present study, alkalinity-induced increased SOD activity with a negative correlation with O$_2^-$ levels (Fig. 5), which indicates that potential SOD activity of this level might not have been efficient enough to neutralize the superoxide radicle. The current study demonstrated higher accumulation of H$_2$O$_2$ even after enhanced activities of the AsA-GSH cycle enzymes, proposing that accumulation of H$_2$O$_2$ exceeded ROS-scavenging potential in salt stressed maize plants (Figs 5 and 6). However, application of JA modulates the AsA-GSH glutathione cycle differentially, by maintaining APX, DHAR, and MDHAR activities above the untreated control level (Fig. 6). Furthermore, addition of JA and SA induced improvement in GR activity paid the AsA-GSH glutathione cycle differently, by maintaining APX, DHAR, and MDHAR activities above the untreated control level (Fig. 6). Furthermore, addition of JA and SA induced improvement in GR activity paid.
the control (Fig. 7A,B). Moreover, an ineffective activity of Gly II might also entrap GSH thereby resulting in 5-lactoylglutathione accumulation, which is highly cytotoxic. However, exogenous application of JA exhibited efficient results on detoxification of MG, as evident by a correlation between reduced MG concentration and amplifying Gly enzyme activities, which thereafter protected cells from MG induced toxicity. Rahman et al. also reported the boosted activities of Gly enzymes (Gly I and II), and enzymatic antioxidants (APX, GPX, and GST) provided efficient salt tolerance in rice plants. Our results advocated that JA might improve maize plant tolerance to salt stress by harmonizing the biochemical activities of the enzymatic and non-enzymatic antioxidants and Gly systems to alleviate alkalinity-induced MG and ROS toxicity by decreasing the MG content and Na\(^+\) influx and K\(^+\) efflux through NSCC and GORK channels and increased nutrient uptake.

In conclusion, we found that supplementation of JA might be an efficient approach for successful tolerance of maize plants under alkaline stress based on the following motives. JA enhances photosynthetic potential by protecting pigments. Second, it maintains water balance and protects cells from oxidative bursting. Third, it diminishes oxidative injury by regulating antioxidant and GSH based Gly systems to detoxification of MG. Thus, our results establish a solid foundation that pretreatment of low dose JA in conveying agricultural land affected with salinity under cultivation will be a sustainable approach to enhance the crop yield.

**Methods**

**Plant growth and treatments.** Plants were grown under controlled conditions—temperature during day/night, 28/24°C; light with photosynthetic photon-flux density, 200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\); and humidity, 65% in a plant growth chamber. Seeds of Z. mays were surface sterilized with 0.1% mercuric chloride for 6 min and then rinsed five times with distilled water. The surface sterilized seeds were then primed with (i) distilled water and (ii) 10 \(\mu\)M JA for 12 h. JA was dissolved in 100% ethanol and then diluted to 10 \(\mu\)M for seed priming. Seed priming was done by soaking the seeds in 10 \(\mu\)M solution of JA and DW for control, for 12 hrs, thereafter air dried. The air-dried seeds of both groups were sown in plastic pots (five seeds/pot) filled with 3 kg of peat, perlite, and sand (1:1:1, v/v/v). After germination, pots were maintained with three seedlings per pot. The pots were arranged in a completely randomized design with three replications. Fifteen days after sowing, the JA primed and non-primed plants were exposed to 0 mM (control), 100 mM, and 150 mM Na\(_2\)CO\(_3\) solution for an additional 10 days (25-day-old seedlings) in the specified conditions. Twenty-five days after sowing, maize plants were harvested to determine various physiological and biochemical responses.

The treatments used are given below:

- 0 mM (control)
- 100 mM Na\(_2\)CO\(_3\)
- 150 mM Na\(_2\)CO\(_3\)
- 0 mM (control) + JA
- 100 mM Na\(_2\)CO\(_3\) + JA
- 150 mM Na\(_2\)CO\(_3\) + JA

**Estimation of plant growth, biomass yield, and total chlorophyll.** The root and shoot lengths were measured using a manual scale. For the measurement of DW seedlings were dried in oven at 70°C for 48 h and then weighed. For the measurement of total chlorophyll of leaves, a 0.5 g leaf sample was homogenized in 5 mL acetone (80% v/v), followed by centrifugation at 10,000 \(\times\) g for 8 min, after which the optical density was measured at 663 and 653 nm for Chl \(a\) and Chl \(b\), respectively, using a spectrophotometer (Beckman 640 D, USA) by following the method of Arnon.

**Analysis of LRWC and proline, total soluble protein, and soluble sugar contents.** For analysing LRWC, fresh weight of leaves was measured after which the leaves were immediately placed between two pieces of filter paper and immersed in double distilled water for 24 h. After removing excess water by paper towel the turgid weight was measured by following the method of Barrs and Weatherley.

To estimate the proline content the method of Bates et al. were employed. Freshly harvested leaf samples (0.5 g) were homogenized with 5 mL (3%) aqueous sulfosalicylic acid. The homogenate was then centrifuged at 11,500 \(\times\) g for 12 min. The supernatant (1 mL) was thoroughly mixed with 1 mL glacial acetic acid and 1 mL acid ninhydrin. The reaction mixture was then boiled at 100°C for 1 h and cooled to stop the reaction. The red colour that developed was removed with 2 mL toluene, and the optical density of the chromophore was measured at 520 nm using a spectrophotometer (Beckman 640 D, USA). Proline concentration was determined using a calibration curve of known proline concentrations.

For the estimation of total protein content, Lowry et al. were used and the optical density was recorded at 595 nm using a spectrophotometer (Beckman 640 D, USA) using bovine serum albumin as the control. For the estimation of TSSs, the absorbance was measured at 485 nm using a spectrophotometer (Beckman 640 D, USA) following the method of Dey.

**Analysis of Na content and accumulation in the seedling roots and leaves.** For the estimation of Na and K content, the separately harvested root and leaf samples were thoroughly washed with double distilled water to eliminate Na and K ions that might be adhering to the surface. The 0.1 g sample of oven dried (80°C for 48 h) tissue was ground and digested with a HNO\(_3\):HClO\(_4\) (5:1 v/v) mixture at 80°C until the yellow colour van-ished. The content of Na and K in roots and leaves was analysed by flame atomic absorption spectrophotometry (Z-5000; Hitachi, Japan).
Measurement of lipid peroxidation, H₂O₂ content, and % EL. Hydrogen peroxide (H₂O₂) was extracted and its content was determined after reaction with 0.1% TiCl₄ in 20% H₂SO₄ Mostofa and Fujita. For the measurement of lipid peroxidation the method of was followed. The production of MDA was measured, and the absorbance difference at 600 nm was detected using an extinction co-efficient of 155 mM⁻¹ cm⁻¹. Leaf EL was estimated by using the method of and the following formula:

\[
EL(%) = \frac{(EC_E/EC_C)}{100}
\]  

Measurement of non-enzymatic antioxidants. Maize leaves (0.5 g) were homogenized in 3 mL ice-cold 5% meta-phosphoric acid containing 1 mM ethylenediaminetetraacetic acid (EDTA) using a mortar and pestle and centrifuged at 11,500 × g for 12 min at 4°C. For the estimation of the total AsA concentration, the oxidized fraction was reduced by 0.1 M dithiothreitol. Reduced and total AsA levels were assayed at 265 nm in 100 mM K-phosphate buffer (pH 7.0) with 1.0 U ascorbate oxidase (AO) Dutilleul et al. DHA content was calculated by subtracting the reduced AsA amount from the total AsA content. The reduced GSH, GSSG, and total glutathione (GSH + GSSG) content were measured using the proposed method of Griffiths. GSSG content was estimated after eliminating GSH by 2-vinylpyridine derivatization. GSH concentration was measured after deducting the value of GSSG from the total GSH content.

Extraction and enzymes assays. Fresh maize leaves (1 g) were homogenized in 100 mM Tris-HCl in presence of 5 mM DTT (dithiothreitol), 10 mM MgCl₂, 1 mM EDTA, 5 mM magnesium acetate, 1.5% polyvinylpyrrolidone (PVP-40), 1 mM phenylmethylsulfonyl fluoride, and 1 µg mL⁻¹ aprotinin. The homogenate was centrifuged at 11,500 × g for 20 min at 4°C and the resulting supernatants were used to estimate enzyme activities.

The activity of lipid peroxidase (LOX, EC 1.13.11.12) was estimated by observing the increase in absorbance at 234 nm using linoleic acid as the substrate solution Doderer et al. The activity of SOD (EC 1.15.1.1) was determined based on a xanthine-xanthine oxidase system El-Shabrawi et al. The reaction mixture contained 50 mM K-phosphate buffer, 2.24 mM nitroblue tetrazolium, 0.1 U CAT, 0.1 U xanthine oxidase, 2.36 mM xanthine, and enzyme extract. The activity of SOD was expressed as units of enzyme required to constrain 50% photoreduction of nitroblue tetrazolium min⁻¹ mg⁻¹ protein.

CAT (EC 1.11.1.6) activity was estimated according to the method of Aebl and the absorbance was measured at 240 nm. For the measurement of APX activity, the decrease in absorbance at 290 nm was monitored as AsA was oxidized Nakano and Asada.

The MDHAR (EC 1.6.5.4) activity was estimated using 1 U AO and the oxidation rate of NADPH at 340 nm was measured Hossain et al. The DHAR (EC 1.8.5.1) activity was determined by observing the formation of AsA from DHA at 265 nm using GSH Nakano and Asada.

The activity of GR (EC 1.6.4.2) was measured by observing decreased absorbance at 340 nm for GSSG-dependent oxidation of NADPH using the extinction coefficient of 6.2 mM⁻¹ cm⁻¹.

The activity of GST (EC 2.5.1.18) was measured following the method of Hossain et al. with an extinction co-efficient of 3.37 mM⁻¹ cm⁻¹.

The activity of Gly II (EC 3.1.2.6) was estimated according to the method of Mostofa and Fujita et al. by employing an extinction co-efficient of 13.6 mM⁻¹ cm⁻¹.

MG content estimation. For the estimation of MG content, the method described by Wild et al. was employed. Fresh maize leaves (0.5 g) were homogenized in 2.5 mL 0.5 M perchloric acid, incubated on ice for 15 min, and centrifuged at 11,200 × g at 4°C for 10 min; thereafter, 1 mL of supernatant was transferred to a centrifuge tube. Charcoal (10 mg mL⁻¹) was added and maintained at 24°C for 15 min to decolourize. Then, the mixture was further centrifuged at 11,000 × g for 10 min, and saturated K₂CO₃ was added to the supernatant for neutralization. In a final volume of 1 mL, 650 µL neutralized supernatant, 330 µL of 100 mM sodium dihydrogen phosphate buffer (pH 7.0), and 20 µL freshly prepared 0.5 M N-acetyl-L-cysteine were added and incubated for 10 min, after which the optical density was recorded at 288 nm. MG content was calculated using a standard curve of known MG concentrations.

Statistical analysis. Data were subjected to analysis of variance and Duncan’s multiple range test. The values represented are means ± SE (n = 3). Different letters indicate significant differences between treatments at P ≤ 0.05.

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**Author Contributions**

M.A.M., R.J. and P.A. designed the experiment and M.A.M. performed the experiments. R.J., M.A.M., M.N.A. and P.A. analyzed the data and results. M.A.M., R.J., M.N.A. and P.A. wrote the manuscript. All the authors read and approved the final manuscript.

**Additional Information**

**Competing Interests:** The authors declare no competing interests.

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