Exogenous 2-(3,4-Dichlorophenoxy) triethylamine alleviates salinity stress in maize by enhancing photosynthetic capacity, improving water status and maintaining K+/Na+ homeostasis

Lijie Li
Northeast Agricultural University
Wanrong Gu (wanronggu@163.com)
Northeast Agricultural University https://orcid.org/0000-0001-9039-7520
Liguo Zhang
Heilongjiang Academy of Agricultural Sciences
Congfeng Li
Chinese Academy of Agricultural Sciences
Xichang Chen
Heilongjiang Academy of Agricultural Sciences
Chunrong Qian
Heilongjiang Academy of Agricultural Sciences
Zhenhua Wang
Northeast Agricultural University
Wenhua Li
Heilongjiang Academy of Agricultural Sciences
Shiyu Zuo
Northeast Agricultural University
Shi Wei
Northeast Agricultural University

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Abstract

Background: Soil salinity restricts plant growth and productivity. 2-(3,4-dichlorophenoxy) triethylamine (DCPTA) can alleviate salinity stress in plants. However, the mechanism of DCPTA-mediated salinity tolerance has not been fully clarified. We aimed to investigate its role in enhancing photosynthetic capacity, improving water status, maintaining K^+/Na^+ homeostasis and alleviating salinity stress in maize (*Zea mays* L.).

Results: In present study, maize seedlings were grown in nutrient solutions with a combination of NaCl (0, 150 mM) and DCPTA (0, 20, 100, and 400 μM). And photosynthesis, water status, ion homeostasis and the expression of genes involved in ion uptake and transport were evaluated in the maize seedlings. The results demonstrated that DCPTA alleviated the growth inhibition of maize seedlings exposed to salinity stress by increasing the net photosynthetic rate (P_n) and the quantum efficiency of photosystem II (PSII) photochemistry. DCPTA improved the root hydraulic conductivity, which help maintained the water status. A relatively high K^+ concentration but a relatively low Na^+ concentration and the Na^+/K^+ ratio were observed in the presence of DCPTA under salinity stress. Additionally, DCPTA altered the expression of four genes (*ZmSOS1*, *ZmHKT1*, *ZmNHX1* and *ZmSKOR*) that encode membrane transport proteins responsible for K^+/Na^+ homeostasis.

Conclusions: DCPTA improved the salinity tolerance of maize may be associated with enhanced photosynthetic capacity, maintenance of water status and altered expression of genes involved in ion uptake and transport.

Background

Soil salinization is one of the most severe adverse environmental factors limiting agricultural development [1, 2]. Approximately 830 million hectares (ha) of land (approximately 20% of the cultivated land area worldwide) is impacted by soil salinization [3-5], and an annual worldwide loss of US$12-27.3 billion occurs due to lost crop production [6]. It is expected that in 2050, the global population will surpass 9.1 billion, necessitating another 70% increase in food production to ensure food security [7]. Soil salinization will be the major obstacle in the way of achieving this goal [8].

Excess salt in the soil impedes plant growth and development by a multitude of mechanisms, including alterations to water relations within the plant (occurring within minutes) [4, 9], and ion deficiency or toxicity (occurring over several hours to days and weeks) [10, 11]. The initial exposure of salinity-sensitive plants to salinity causes water stress because plant root access to soil water is reduced by the increased osmotic strength of the soil solution [10, 12]. Osmotic stress impairs cell water relations, inhibits cell expansion and division and decreases stomatal aperture and transpiration [12, 13]. Stomatal closure limits the diffusion of atmospheric CO_2 to the site of carboxylation and causes the stomatal limitation of photosynthesis, consequently decreasing growth rates [13]. Salinity-induced decreases in photosynthetic efficiency are often associated with the inhibition of photosystem II (PSII), which plays a central role in light energy conversion and electron transport [14]. Hydraulic conductivity (Lp) refers to the ease with which water can flow from one location to another and therefore influences the rate of water movement [13]; Lp can be used to indicate the ability of plant roots to absorb water [15]. Under salinity stress, the maintenance of Lp is an essential part of the adaptation process that helps to restore plant growth [13].

During long-term exposure to salinity, plants undergo ionic stress, particularly due to sodium chloride, which causes plant nutritional imbalance and oxidative stress, with severe consequences for plant growth, development and survival [4, 10, 12, 16]. Excess Na^+ is particularly deleterious to plants because it competes with K^+ for metabolic processes required for K^+, leading to enzyme inactivation, plant nutritional imbalance, protein degradation, leaf photochemistry inhibition and oxidative stress [17]. All of these effects synergistically inhibit plant growth and development [10]. Previous studies have demonstrated that the K^+/Na^+ ratio is considered an important indicator for evaluating the salinity resistance of various plant species [4, 18]. The regulation of several genes encoding membrane transport proteins involved in Na^+ and/or K^+ uptake, translocation or compartmentalization is an important strategy for plants to address excessive Na^+ accumulation and K^+ deficiency. The plasma membrane Na^+/H^+ antiporter (*SOS1*, located in the plasma membrane) and the tonoplast (Na^+, K^+)/H^+ antiporter (*NHX1*, located in the vacuolar membrane) are involved in a plant's salt tolerance by compartmentalizing Na^+ into the vacuoles, and/or effluxing Na^+ from cells across membranes to maintain non-toxic levels of cytosolic Na^+ [9, 19]. The high-affinity potassium transporter 1 (*HKT1*) is localized primarily in the root stele and is responsible for unloading Na^+ from the xylem to control Na^+ long-distance transport to the shoots and to regulate root-to-shoot Na^+ distribution [20, 21]. Moreover, the outward-rectifying K^+ channel (*SKOR*) mediates K^+ secretion from root cells into the xylem and K^+ transport to the shoots [22].

The use of biostimulants, which are kinds of natural or synthetic small bioactive molecules derived from human- or animal-related industrial processes, is considered an effective measure to ameliorate growth inhibition induced by salinity or to improve plant resistance to salinity stress [10, 23]. The tertiary amine bioregulator 2-(3,4-dichlorophenoxy) triethylamine (DCPTA) represents a class of highly bioactive, low-molecular-weight amine compounds that have a significant regulatory effect on crop growth and development [24]. Previous studies have shown that DCPTA can improve the dry weight (DW) of tomato plants [25]; increase the root DW and leaf area of beet, radish [26], and maize [27]; and enhance ribulose-1,5-bisphosphate activity and increase the size of chloroplasts in the leaves of sugar beet (*Beta vulgaris* L.) [28]. DCPTA can also enhance CO_2 fixation in cotton, promote chlorophyll biosynthesis in guayule [29] and stimulate carotenoid biosynthesis in citrus [30]. Suitable concentrations of DCPTA can improve the net photosynthetic rate (P_n) in maize (*Zea mays* L.) [31] and have anti-senescent properties, as demonstrated by the slowing of chlorophyll degradation in bean (*Phaseolus vulgaris* L.) leaf discs in darkness [32]. Moreover, DCPTA can increase the ability of crops to adapt to stress and improve stress resistance [33-36]. Xie et al. [35] showed that spraying DCPTA can increase the leaf relative water content (LRWC) and promote water uptake, as indicated by increased Lp, which may be due to improvements in root growth, increased photosynthetic capacity associated with the increased chlorophyll content, greater photosynthetic C_4 enzyme activity and reduced damage to chloroplasts under drought stress, thereby increasing the drought tolerance of maize seedlings. DCPTA can also increase tolerance to low-temperature stress by increasing the maximum quantum efficiency of PSII photochemistry (F_v/F_m) and the chlorophyll content, which effectively was shown to protect the photosynthetic system of maize leaves under low-temperature stress [33].

Maize is one of the most important cereal food crop species grown worldwide and provides raw materials for industry [37]. The area of maize production is the largest of food crop species, and its main planting areas are in irrigated agricultural areas in both arid and semi-arid regions. However, intensive irrigation results in high salinity levels [38]. The salinity-induced disturbance of nutritional status in growing cells inhibits maize growth and ultimately yield [39]. As maize is considered a moderately salinity-sensitive plant species [40], few salinity-tolerant maize cultivars have been commercialized [41]. Therefore, soil salinity stress has become one of the most serious threats to sustainable maize production [40]. A previous study showed that addition of DCPTA can alleviate reductions in root DW caused by salinity stress [34]. However, little is known about how DCPTA applications and doses can trigger adaptation to NaCl stress via morpho-physiological responses and ion homeostasis, particularly the molecular mechanisms involved. Therefore, the aims of this study were the following: (1) to determine the effects of DCPTA on the leaf photosynthetic capacity and chlorophyll fluorescence of maize exposed to salinity stress, (2) to evaluate the responses of the leaf water status (leaf water potential (Ψω) and LRWC) and Lp of maize in response to applications of DCPTA under salinity stress, and (3) to explore the regulation of the concentration of Na^+ and K^+ and the Na^+/K^+ ratio in the leaves and roots of maize and the expression of four genes (*ZmSOS1*, *ZmHKT1*, *ZmNHX1*, and *ZmSKOR*) that encode transport proteins responsible for Na^+ and/or K^+ uptake, translocation or compartmentalization in response to DCPTA in the leaves and roots of maize subjected to salinity stress. This systematic investigation will provide additional information for an improved understanding of the regulatory mechanisms of DCPTA-mediated salinity stress tolerance in maize.

Results

Plant growth and root characteristic parameters

The root and shoot DWs of maize were significantly affected by NaCl and DCPTA ($P \leq 0.001$), and the interaction between NaCl and DCPTA application rate had no significant effect on the root and shoot DWs (Table 1). As shown in Fig. 1 and Fig. 2a, DCPTA has a dose-dependent effect on growth. Under non-stressed conditions, low doses of DCPTA (20 μM and 100 μM) significantly increased the root and shoot DWs, while a relatively high concentration of DCPTA (400 μM) had no significant effect on plant growth. Compared with non-salinity conditions, salinity stress significantly inhibited plant growth ($P \leq 0.05$), and the root and shoot DWs were reduced by 40.0% and 30.1%, respectively. However, the application of various concentrations (20-400 μM) of DCPTA obviously alleviated the salinity stress-induced reduction in the root and shoot DWs, whereas 100 μM DCPTA appeared to be the most effective concentration for alleviating salinity stress. Under salinity-stressed conditions, compared with those of untreated maize, the root and shoot DWs of maize treated with 100 μM DCPTA significantly improved by 33.4% and 26.2%, respectively. Both the low and high doses of DCPTA were less effective at promoting plant growth under salinity stress.

The root length, surface area and volume of maize were significantly affected by the application of NaCl and DCPTA ($P \leq 0.001$), and the interaction between NaCl and DCPTA application rate significantly affected the root length of maize (Table 1). As shown in Fig. 2b, c and d, compared with the control conditions, the salinity stress significantly decreased the total root length, root surface area and root volume by 47.7%, 46.4% and 37.5%, respectively. The NaCl-induced decreases in root length and root surface area were alleviated by applications of DCPTA at 20 and 100 μM – more markedly with the latter. Furthermore, the NaCl-induced decreases in root volume were alleviated by applications of DCPTA at 100 and 400 μM – more remarkably with the former.

LRWC, leaf Ψω and Lp

The LRWC, leaf Ψw and Lp were significantly affected by DCPTA and NaCl concentration; the interaction between DCPTA and NaCl concentration was significant (Table 1). Under the control conditions, application of DCPTA had no significant effect on leaf Ψω or LRWC; however, compared with the control, 100 μM DCPTA stimulated significant increases in Lp, which increased by 14.7% (Fig. 3). Compared with the non-stressed conditions, the salinity stress reduced the leaf Ψω, LRWC and Lp by 76.43%, 45.56% and 57.4%, respectively. The NaCl-induced decreases in leaf Ψω and Lp were alleviated in plants treated with DCPTA at 100 μM and 400 μM – more markedly with the former; however, the reduction in LRWC was alleviated in plants treated with DCPTA (20-400 μM), especially with DCPTA at 100 μM. Compared with salinity stress alone, the application of 100 μM DCPTA increased the leaf Ψω, LRWC and Lp by 59.8%.

Chlorophyll content and gas exchange parameters

The contents of Chl a and Chl b were significantly affected by DCPTA and NaCl concentration, and the interaction between NaCl and DCPTA significantly affected the contents of Chl a and Chl b (Table 2). Under the control conditions, applications of DCPTA at 20 μM and 100 μM stimulated significant increases in the contents of Chl a and Chl b (Fig. 4). Compared with the non-stressed conditions, NaCl significantly affected Chl a and Chl b in maize and reduced their contents by 59.0% and 64.1%, respectively. Under salinity stress conditions, the 20 μM and 100 μM DCPTA-treated plants presented a greater increase in the contents of both Chl a and Chl b than did the plants not treated with DCPTA, and the increase was greater with 100 μM than with 20 μM DCPTA. Compared with salinity stress alone, 100 μM DCPTA increased the contents of Chl a and Chl b by 56.8% and 84.3%, respectively.

The P_n, C_i, g_s and T_r were significantly affected by DCPTA and NaCl concentration. The interactive effects of DCPTA and NaCl concentration on the P_n were significant (Table 3). As shown in Fig. 5, the P_n increased by 7.9% and 12.9% in plants treated with 20 μM and 100 μM DCPTA, respectively, compared with the plants treated without DCPTA under non-stressed conditions. NaCl markedly decreased the P_n in maize leaves. Under stressed conditions, compared with that in non-DCPTA-treated plants, the P_n in plants treated with DCPTA at concentrations of 20 μM and 100 μM increased by 19.1% and 40.7%, respectively. Similarly, g_s and T_r were significantly reduced by salinity stress, and applications of DCPTA at 20 μM and 100 μM alleviated these adverse effects in the leaves of plants under stressed conditions. In contrast, salinity stress significantly stimulated an increase in C_i, but this induction was attenuated by pretreatment with DCPTA at 100 μM.

Chlorophyll fluorescence parameters

The F_v/F_m, ΦPSII, qP and NPQ were significantly affected by DCPTA and NaCl concentration. The interactions between DCPTA and NaCl concentration were significant (Table 2). Under non-stressed conditions, there was no effect of DCPTA on the obtained chlorophyll fluorescence parameters (Fig. 6). Compared with those in the control, the F_v/F_m, ΦPSII and qP in response to treatment with 150 mM NaCl markedly declined, and the NPQ increased significantly. The NaCl-induced decreases in F_v/F_m and ΦPSII were alleviated by 100 μM and 400 μM DCPTA, especially with the former, while the decreases in qP were alleviated by applications of DCPTA at 20 μM and 100 μM – more markedly with the latter. Addition DCPTA significantly suppressed the NPQ, and the effect was greater with 100 μM DCPTA than with 400 μM DCPTA or 20 μM DCPTA under salinity stress conditions. On the 5th day, compared with the salinity stress treatment, the DCPTA treatment at 100 μM markedly increased the F_v/F_m by 31.1%, the ΦPSII by 49.5% and the qP by 45.2% and decreased the NPQ by 20.1%.

Na^+ concentration, K^+ concentration, the Na^+/K^+ ratio, and the shoot/root Na^+ ratio

NaCl and DCPTA significantly affected the contents of Na^+ and K^+ and the Na^+/K^+ ratio in both the shoots and roots of maize plants ($P \leq 0.001$). In addition, the interaction between NaCl and DCPTA significantly affected the contents of Na^+ and K^+ and the Na^+/K^+ ratio in both the shoots and roots of maize plants (Table 3). As shown in Fig. 7, 150 mM NaCl significantly affected the concentrations of Na^+ and K^+ in both the roots and shoots of maize plants. In response to the 150 mM NaCl treatment, the K^+ concentrations in the roots and shoots decreased by 37.5% and 51.6%, respectively; the Na^+ concentrations in the roots and shoots increased by 24.6% and 51.5%, respectively; and the Na^+/K^+ ratio in the roots and shoots increased by 24.6% and 51.5%, respectively. DCPTA altered the Na^+ and K^+ accumulations in maize plants under both non-stressed and stressed conditions. Under non-stressed conditions, the addition of DCPTA (20-400 μm) increased the K^+ concentrations in both the roots and shoots of plants; however, these applications did not increase the Na^+ concentrations. Additionally, 100 μM DCPTA appeared to be the most effective concentration for increasing K^+ concentrations and decreasing Na^+ concentrations under salinity stress. DCPTA application significantly affected the Na^+/K^+ ratio in both the roots and shoots of salinity-stressed plants. Under salinity stress, compared with those of non-DCPTA-treated plants, the Na^+/K^+ ratios of the shoots of plants treated with 20, 100 and 400 μM DCPTA decreased by 146.3, 221.6, and 66.5%, respectively, and the ratio of Na^+/K^+ in the roots decreased by 61.1, 71.3, and 22.3%, respectively.

Gene expression

NaCl significantly affected the expression of *ZmSOS1*, *ZmNHX1* and *ZmHKT1* in both the shoots and roots and *ZmSKOR* in the roots of maize plants ($P \leq 0.001$). DCPTA and the interaction between NaCl and DCPTA significantly affected the expression of *ZmSOS1*, *ZmHKT1* and *ZmSKOR* in both the shoots and roots and Zm*NHX1* in the shoots of maize plants (Table 3). As shown in Fig. 8a, the expression of *ZmSOS1* was significantly upregulated in both the roots and shoots of the different plants treated with all the different DCPTA treatments; moreover, compared with that in the untreated plants, the expression in the treated plants was greater under salinity-stressed conditions. In the shoots, DCPTA application significantly increased *ZmHKT1* expression under both stressed and non-stressed conditions (Fig. 8b). In the roots, DCPTA application decreased *ZmHKT1* expression slightly under non-stressed conditions but notably under stressed conditions. Expression of *ZmNHX1* was significantly upregulated in the shoots in response to application of DCPTA; moreover, compared with that in untreated plants, the expression in the treated plants increased under salinity-stressed conditions. *ZmSKOR* expression was upregulated in the roots and shoots of plants treated with DCPTA under both stressed and non-stressed conditions.

Discussion

The harmful effects caused by salinity stress involve various physiological and biochemical mechanisms related to plant growth and development [4, 42]. In this study, salinity stress significantly inhibited the photosynthesis and biomass accumulation of maize seedlings. Research has shown that there is a two-phase growth response to salinity: the growth is first reduced by the decrease in soil water potential and then by salt injury to the older leaves, because of a rapid rise in salt concentration in cell walls of cytoplasm when the vacuoles can no longer sequester incoming salts (Munns 1993). However, the addition of DCPTA, particularly at 100 μM, significantly alleviated NaCl-induced inhibition of photosynthesis and biomass production, which suggests that DCPTA mitigates salinity stress in maize seedlings possibly by improving water status and maintaining K^+/Na^+ homeostasis. The beneficial influence of DCPTA on photosynthesis and biomass production has also been reported for both soybean [43] and maize [35] under drought stress and for maize under low-temperature stress [33, 36].

In this study, we observed that T_r and g_s significantly decreased in response to NaCl stress. In addition, salinity stress significantly increased the C_i, which may be due to decreased CO_2 assimilation induced by photosystem photo-oxidation or damage and the inactivation of the photosynthesis enzymes under salinity stress [13]. Such results indicate that non-stomatal limitations were the primary cause of the decrease in the P_n of plants grown under salinity conditions (Farquhar and Sharkey, 1982). However, the reduction in g_s and T_r and the increase in C_i induced by salinity stress were alleviated by DCPTA in a dose-dependent manner, which is consistent with the results of a previous study by Xie et al. [35] in which DCPTA exerted positive effects on plant photosynthetic capability. The lower reduction in g_s and T_r in DCPTA-treated plants than in non-DCPTA-treated plants suggests CO_2 diffusion through the stomata was less diminished; hence, the water status may be better in DCPTA-treated plants. Moreover, the lower C_i in DCPTA-treated plants might be a consequence of the ameliorated water status, the cytoplasmic Na^+ homeostasis and the relative stability of the Na^+/K^+ ratio, reducing the toxicity of Na^+ in the cytoplasm to organelles such as the chloroplast, leading to a lower metabolic limitation of CO_2 assimilation in these plants compared with non-DCPTA-treated plants. Thus, alleviation of non-stomatal limitation by DCPTA contributed to improvements in photosynthetic capacity under salinity stress. In this study, salinity significantly decreased the chlorophyll contents, F_v/F_m, ΦPSII and qP, which caused a reduction in the light-absorbing efficiency and electron transport within PSII. However, these reductions in the chlorophyll contents, F_v/F_m, ΦPSII and qP were alleviated with applications of DCPTA at a relatively low level under salinity conditions, which implies that, compared with the untreated plants, the plants treated with DCPTA had a less impaired PSII, a greater activity of membrane-associated electron carriers in excited PSII reaction centres and a greater quantum efficiency of PSII photochemistry under salinity conditions. In addition, the increase in NPQ indicated that excitation energy was excessive for the capacity of electron transport in plants exposed to salinity. However,

exogenous DCPTA effectively diminished the increase in NPQ induced by NaCl, which indicated that DCPTA could promote the use efficiency of absorbed light in photochemical processes, with minimal thermal dissipation and fluorescence emissions [33, 35]. The improvement in PSII efficiency in the DCPTA-treated plants possibly resulted from an alleviation of oxidative damage to photosynthetic apparatus under DCPTA pretreatment, because the accumulation of Na in the leaves was reduced, and K accumulation in leaves was increased when maize seedlings were pretreated with DCPTA. Thus, these results indicated that, DCPTA improved PSII efficiency, which ultimately increased the photosynthetic capability and salinity tolerance of maize.

The promotion of increased plant biomass and photosynthetic capacity due to the effects of DCPTA can also be attributed to an amelioration of water status. In this study, DCPTA alleviated the decrease in water status under salinity stress, as indicated by the LRWC and leaf Ψω, which may be attributed to the dynamic balance maintained between plant root water absorption and leaf transpiration [13]. Moreover, the relatively low reduction in the LRWC and leaf Ψω in the stressed plants treated with DCPTA was consistent with the relatively low reduction in g_s and T_r in those plants. Given that the beneficial effect of DCPTA on plant water status is due to an increase in root water absorption, which is largely achieved by improved root growth (which is associated with the root DW, length, surface area and volume) and Lp, not a decrease in water loss due to the increase in g_s and T_r. These results suggest that DCPTA increased the root water uptake ability and thus improved the water status of maize plants under salinity stress.

Salts absorbed by the roots are transported to the shoots over long distances in the transpiration stream, and leaves are the main location of Na^+ accumulation in most plants [4]. In this study, the addition of DCPTA induced substantial decreases in the Na^+ concentration in the shoots, which was accompanied by a decreased shoot/root Na^+ ratio under salinity stress. To reduce cytoplasmic Na^+ concentrations, plants have evolved various adaptive mechanisms, including restricting Na^+ uptake from the soil solution, extruding excessive Na^+ and vacuolar partitioning of Na^+ to decrease Na^+ accumulation in the cytosol [44]. In this study, under salinity stress conditions, the expression of *ZmSOS1* in the roots and shoots was upregulated, and DCPTA further upregulated the expression of *ZmSOS1* in the roots; in turn, this upregulation decreased the Na^+ influx from the external solution into the cytosol or promoted Na^+ export to the apoplastic space [11, 45]. Therefore, DCPTA may increase the capability of plants to extrude Na^+ into soil solution and/or mitigate the toxic effects of Na^+, as the toxicity of Na^+ is small in the apoplastic space [46]. DCPTA increased shoot *ZmHKT1* expression under salinity stress, which facilitated Na^+ recirculation into the xylem and Na^+ allocation to the roots. In contrast, DCPTA decreased *ZmHKT1* expression in the roots, which reduced Na^+ loading into the xylem and subsequently transported Na^+ to sensitive photosynthetic tissues, suggesting that, compared with untreated plants, plants treated with DCPTA may be more capable of restricting Na^+ accumulation in sensitive tissues. *HKT* transporters have been shown to mediate the translocation of Na^+ from the roots to the shoots by retrieving Na^+ from the root-to-shoot xylem sap, which represents a strategy to avoid the toxic effects of the photosynthetic apparatus [9, 42]. In this study, the dysregulation of *ZmHKT1* under salinity stress and in response to DCPTA was correlated with the lower shoot/root Na^+ ratio in the plants treated with DCPTA compared with the untreated plants. Increasing the expression of *ZmSOS1* in the roots and *ZmHKT1* in the shoots while decreasing the expression of *ZmHKT1* in the roots contributed to decreased Na^+ concentration in the shoots of the plants treated with DCPTA and could therefore be a key consequence of the effect of DCPTA on plants exposed to salinity stress. In addition, a recent study showed that Na-acclimated maize plants have improved vacuolar Na^+ sequestration ability in their leaves and can accumulate relatively large amounts of Na^+ in those organs without any detrimental effects on photosynthetic capacity [47]. It has been proposed that *NHX* functions in Na^+ compartmentalization in the vacuole and efflux of Na^+ from cells [4], and the upregulation of *NHX* in transgenic plant species such as *Brassica napus* [48], poplar [49] and tomato [50] has been shown to increase plant salinity tolerance. Moreover, a previous study showed that salinity-tolerant maize hybrids exhibited higher expression of *ZmNHX1* than did salinity-sensitive hybrids exposed to salinity stress [51]. In this study, *ZmNHX1* expression was upregulated in both roots and shoots of plants under salinity stress, and further upregulated in the shoots of DCPTA-treated plants under salinity conditions. The upregulation of *ZmNHX1* expression in the shoots in response to DCPTA was responsible for increased Na^+ compartmentalization in the leaf vacuoles, which was beneficial for reducing the Na^+ toxicity in the cytosol, improving the osmolarity in the vacuole, and concomitantly enhancing plant salinity tolerance [52].

Owing to the similar hydration radius of K^+ and Na^+, excess Na^+ osmoticum obviously competes for K^+ entry into the symplast at transport sites, which causes a reduction in K^+ concentration and alters the Na^+/K^+ ratio under salinity stress [18]. In this study, DCPTA significantly increased the K^+ concentration and decreased the Na^+/K^+ ratio in the plants under salinity stress, which was presumably crucial for salinity tolerance [4]. It has been shown that the *SKOR* channel influences the xylem loading of K^+ [16]. In this study, DCPTA significantly upregulated the expression of *ZmSKOR* in plants exposed to 150 mM NaCl, which may have contributed to K^+ release into the xylem for transport towards the shoots [53, 54] and was correlated with increased K^+ concentration in plants treated with DCPTA in this study. Moreover, the upregulation of *ZmSKOR* in combination with *ZmSOS1* and *ZmHKT1* in the shoots as well as the downregulation of *ZmHKT1* in the roots of plants treated with DCPTA can account for the reduced Na^+/ K^+ ratio of plants under salinity stress.

Conclusion

In conclusion, we have demonstrated that DCPTA increased the salinity stress tolerance of maize seedlings in a dose-dependent manner. DCPTA increased the photosynthetic capacity of maize plants under salinity stress by regulating stomatal movement and improving both light energy absorption and electron transport in PSII, which may be associated with improved water status and maintained K^+/Na^+ homeostasis. Under saline conditions, the increase in Lp in response to DCPTA, indicating increased root water uptake ability, was beneficial for maintaining leaf water status and contributed to water transport to specific tissues and CO_2 diffusion across the plasma membrane. In addition, the plants treated with DCPTA were capable of extruding Na^+ out of the root cells, unloading Na^+ from the xylem, sequestering Na^+ in the vacuole, and translocating K^+ to the shoots, all of which are related to the upregulation of *Zm*SOS1 and *SKOR* expression in both the shoots and roots and *ZmHKT1* and *ZmNHX1* expression in the shoots and the downregulation of *ZmHKT1* expression in the roots under salinity stress. The improved photosynthesis, water status, and K^+/Na^+ homeostasis contributed to improved growth and salinity stress tolerance (Fig. 9); thus, applications of low levels of DCPTA can be a sustainable approach to increase crop yields under salinity stress.

Material And Methods

Plant materials and stress treatments

Seeds of maize (cultivar ZD958, a local commonly planted cultivar) (Beijing De Nong Seed Industry Co., Ltd., Beijing, China) were surface sterilized for 10 min with mercuric chloride (0.2%) and then rinsed abundantly with distilled water. Afterward, the seeds were soaked in distilled water for 12 h and then germinated on double-layered filter paper moistened with distilled water at 28 °C for 2 days in the dark. The germinated seeds were sown in quartz sand in a climate chamber (HPG-280HX, Harbin Donglian Electronic Technology Development Co., Ltd., Harbin, China) whose temperature was set to 28 °C/18 °C, corresponding to a 12 h/12 h (day/night) photoperiod; the light intensity in the chamber was 350-450 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the relative humidity was 50-60%. Two-leaf-stage seedlings of uniform size were transferred to plastic pots (40.5 cm × 32.5 cm × 12 cm, 12 plants per pot) containing 1/2-strength Hoagland nutrient solution (pH 6.5). The nutrient solution was changed every three days to avoid nutrient depletion. The nutrient solution comprised the following components: 2.5 mM $Ca(NO_3)_2$, 1.0 mM K_2SO_4, 0.2 mM KH_2PO_4, 0.6 mM $MgSO_4$, 2.5 mM $CaCl_2$, 0.5 mM NaCl, 1.0 μM H_3BO_4, 2.0 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.005 μM $(NH_4)_6Mo_7O_{24}$, and 200 μM Fe-EDTA. When the seedlings grew to the three-leaf stage, they were treated with the following different nutrient solutions: (1) 1/2-strength Hoagland nutrient solution (CK); (2) 1/2-strength Hoagland nutrient solution + 20 μM DCPTA (20 μM DCPTA); (3) 1/2-strength Hoagland nutrient solution + 100 μM DCPTA (100 μM DCPTA); (4) 1/2-strength Hoagland nutrient solution + 400 μM DCPTA (400 μM DCPTA); (5) 150 mM NaCl in 1/2-strength Hoagland nutrient solution + 0 μM DCPTA (NaCl); (6) 150 mM NaCl in 1/2-strength Hoagland nutrient solution + 20 μM DCPTA (NaCl + 20 μM DCPTA); (7) 150 mM NaCl in 1/2-strength Hoagland nutrient solution + 100 μM DCPTA (NaCl + 100 μM DCPTA); and (8) 150 mM NaCl in 1/2-strength Hoagland nutrient solution + 400 μM DCPTA (NaCl + 400 μM DCPTA). The DCPTA (Zhengzhou Zhengshi Chemical Limited Co., Ltd., China) was applied 2 days before the addition of NaCl in the nutrient solution. The NaCl concentration was gradually increased in 50 mM increments every 8 h to avoid salinity shock. The experiment included 8 treatments with 5 replicates (90 plants in total per treatment). Each replicate included 2 pots of 18 plants (9 uniform plants were selected from the 12 plants per pot). Five days after the salinity level reached 150 mM, the gas exchange, chlorophyll fluorescence parameters, LRWC and leaf Ψω were measured for the second fully expanded leaf, and the root Lp was measured. The plants in the 8 treatments were then collected, frozen immediately in liquid nitrogen and stored at -80 °C until the plant biomass determination and biochemical assays.

In the second experiment, the plants were cultivated in the same manner as described above. After 24 h of salinity treatment (0 and 150 mM NaCl) in combination with DCPTA (0, 20 and 100 μM), the leaves and roots of the maize seedlings of 6 treatments (including five replicates) were sampled by mixing 5 seedlings per replicate, frozen immediately in liquid nitrogen and then stored at -80 °C for RNA sequencing (RNA-seq).

Plant growth, root characteristic parameters and ion concentrations

After 5 days of the treatments, the plants were divided into two parts: roots and shoots. The root characteristic parameters, including the root length, surface area, and volume, were then scanned with a root scanner (MRS-9600TFU2L, Shanghai Microtek Technology Co., Ltd. Shanghai, China). The roots and shoots were subsequently dried in an oven at 80 °C to a constant weight to determine the DW. The dry samples of the roots and shoots were ground into a fine powder and passed through a 1 mm diameter mesh stainless steel sieve. Afterward, 0.1 g of the root and shoot samples was digested with a 5 mL of a HNO_3:$HClO_4$ (5:1 v/v) solution at 80 °C until the sample consisted of only a small white residue. The sample was then brought to 50 mL with deionized water. The contents of K^+ and Na^+ in the roots and shoots were analysed by flame atomic absorption spectrophotometry (Z-2000; Hitachi, Japan).

LRWC

To analyse the LRWC, the second fully expanded leaves were collected and weighed to determine the fresh weight (FW), after which they were immersed in distilled water for 24 h in the dark. The leaves were subsequently gently blotted dry with absorbent paper and weighed to determine their turgid weight (TW). The samples were then dried in an oven at 105 °C for 20 min followed by 80 °C until a constant weight was achieved, at which point the DW was determined. The LRWC was calculated using the following formula: LRWC (%) = [(FW - DW) / (TW - DW)] × 100.

Leaf Ψω

The leaf Ψω of the second fully expanded leaves was determined by the pressure chamber technique (type 3115 pressure chamber, Beijing Huahai Heng Hui Technology Co., Ltd. Beijing, China). The second fully expanded leaves were harvested and inserted immediately into the rubber plug hole of the pressure chamber such that the incision was exposed approximately a few millimetres outside the sealing ring for convenient observation. After a good seal was confirmed, the pressure control valve was rotated to slowly pressurize at 0.05 $MPa \cdot s^{-1}$. When a water film appeared at the incision, rotating of the control valve was stopped, and the pressure value at this time was recorded as the leaf Ψω.

Chlorophyll content

To measure the chlorophyll content in the leaves, a fresh leaf sample (0.5 g) and 5 mL of acetone (80% v/v) were homogenized together in an ice bath. After centrifugation at 10,000 g for 10 min at 4 °C, the absorbance at 645 and 663 nm was monitored spectrophotometrically with a spectrophotometer (UV-5500, Shanghai Yuan Analysis Instrument Co., Ltd., China) to determine the contents of chlorophyll a (Chl a) and chlorophyll b (Chl b), respectively. These contents were calculated in accordance with the equations of Arnon [55].

Gas exchange parameters

The gas exchange parameters (the net photosynthesis rate (P_n), stomatal conductance (g_s), the intercellular CO_2 concentration (C_i) and the transpiration rate (T_r)) were determined with an LI-6400 portable photosynthetic system (LI-COR Inc., USA) at 9:00-11:00 a.m. for the different treatments of maize seedlings. The second fully expanded leaves were used for the assays. During the measurements, the leaf chamber temperature was maintained at approximately 26 °C,

the photosynthetic photon flux density (PPFD) was 800 μmol m^{-2} s^{-1}, the CO_2 concentration was 400 μmol·mol^{-1}, and the relative humidity was 60-70%. Five seedlings per treatment were used to measure the gas exchange parameters.

Chlorophyll fluorescence parameters

Chlorophyll fluorescence was determined via a PAM2000 modulated fluorescence spectrometer (Walz, Germany). After 30 min of dark adaptation, the minimum fluorescence (F_o) was obtained by irradiating the measured light (< 0.05 μmol·m^{-2}·s^{-1}), and the maximum fluorescence (F_m) was measured via a saturated pulse light (0.8 s; 8000 μmol·m^{-2}·s^{-1}). The photosynthetic steady-state fluorescence (F_s) was measured by turning on the actinic light (300 μmol·m^{-2}·s^{-1}), the saturated pulse light (8000 μmol·m^{-2}·s^{-1}) was turned on again to obtain the maximum fluorescence (F_m'), the actinic light was turned off, the far-red light was turned on immediately, and the minimum fluorescence (F_o') under the light was obtained. Other parameters were calculated as follows: the F_v/F_m = (F_m - F_o) / F_m; the photochemical quantum efficiency of PSII (ΦPSII) = (F_m' – F_s) /Fm'; the photochemical quenching coefficient (qP) = (F_m'- F_s) / (F_m'- F_o'); and the non-photochemical quenching (NPQ) coefficient = (F_m – F_m') /F_m' [56].

Root Lp

The determination of Lp was performed according to the method of Nardini et al. [57], with slight modifications. The roots of 5 maize seedlings per treatment were cut and removed. The whole roots were then placed in a pressure chamber, the cut was exposed to the outside of the seal ring by approximately a few centimetres, and a gradually increasing pressure (from 0.1 to 0.4 MPa) was applied to the roots. The sap was collected with pre-prepared, dried absorbent cotton in Eppendorf tubes at each pressure gradient (for 1 min) and then weighed on a precision balance, thereby generating a range of sap flows that represented the whole-plant T_r. The root Lp was calculated according to the following formula: Lp = Jv/P, where Jv (m·s^{-1}) is the flow rate and P (MPa) is the external pressure.

RNA extraction and quantitative RT-PCR

To extract RNA using a Qiagen RNeasy Plant Mini Kit (Shanghai Baili Biotechnology Co., Ltd., Shanghai, China), 0.1 g of root and shoot samples was used. The cDNA was then reverse transcribed using an iScript^{TM} cDNA synthesis kit (Bio-Rad, California, USA) kit. The cDNA was diluted 50-fold, and 2 μL was taken for qRT-PCR analysis. Actin1 was used as an internal reference gene. A relative quantitative analysis was performed using the $2^{-\triangle\triangle CT}$ method. The primers used are listed in Table S1. Five replicates per treatment and 5 technical replicates per plant were included.

Statistical analysis

SPSS 19.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The data were subjected to two-way ANOVA with three sources of variation (NaCl, DCPTA and their interaction), and the differences between the treatment means within each measured parameter were compared via Duncan's multiple range test ($P < 0.05$).

Abbreviations

C_i, intracellular CO_2 concentration; Chl a, chlorophyll a; Chl b, chlorophyll b; DCPTA, 2-(3,4-dichlorophenoxy) triethylamine; DW, dry weight; F_o: minimum fluorescence; F_m, maximal fluorescence yield of the dark-adapted state; F_v/F_m, maximal quantum yield of PSII photochemistry; g_s, stomatal conductance; Jv, sap flow; Lp, root hydraulic conductivity; LRWC, leaf relative water content; NPQ, non-photochemical quenching; P, pressure; P_n, net photosynthesis rate; PSII, photosystem II; PPFD, photosynthetic photon flux density; qP, photochemical quenching coefficient; ROS, reactive oxygen species; T_r, transpiration rate; Ψw, water potential; ΦPSII, effective quantum yield of PSII photochemistry

Declarations

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Authors' contributions

LJL performed the entire experiment and drafted the manuscript. WRG, and SW conceived and designed the experiments. LJL, and SYZ gathered the data and analysed the results. SYZ, CFL, GLZ, CXC and CRQ helped to interpret the results and prepare the manuscript. WRG, SW, ZHW and WHL were in charge of manuscript revisions. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1 Analysis of variance and mean comparisons for plant dry weight (Shoot DW: shoot dry weight; Root DW: root dry weight), leaf water status (RWC: relative water content; Ψw: leaf water potential), root hydraulic conductance (Lp) of maize plants grown under two salinity levels and treated with DCPTA at four rates of application.

Source of variation	Shoot DW ($g \cdot plant^{-1}$)	Root DW ($g \cdot plant^{-1}$)	Root length ($cm \cdot plant^{-1}$)	Root surface area ($cm^2 \cdot plant^{-1}$)	Root volume ($cm^3 \cdot plant^{-1}$)	Ψw (MPa)	RWC (%)	Lp ($10^{-7} \cdot m^3 \cdot m^{-2} \cdot s^{-1}$ MPa^{-1})
NaCl (mM) (N)								
0	0.19a	0.12a	475.64a	120.12a	3.95a	-0.73a	93.34a	11.63a
150	0.13b	0.09b	279.30b	71.97b	2.68b	-1.69b	71.99b	6.00b
DCPTA (µM) (D)								
0	0.14b	0.10	346.20	86.95	3.10	-1.28	78.38	8.03
20	0.17ab	0.11	384.99	101.75	3.24	-1.24	84.52	8.60
100	0.18a	0.11	420.48	103.32	3.60	-1.09	85.38	9.89
400	0.16ab	0.11	358.22	92.16	3.32	-1.23	82.35	8.74
Significance								
NaCl (mM) (N)	***	***	***	***	***	***	***	***
DCPTA (µM) (D)	***	***	***	***	***	***	***	***
N×D	ns	ns	**	ns	ns	**	**	*

ns, *, **, and ***: Not significant or significant at P≤0.05, 0.01, and 0.001, respectively. The different letters within each column indicate significant differences according to Duncan's multiple-range test (P=0.05).

Table 2 Analysis of variance and mean comparisons for chlorophyll content (Chl a: chlorophyll a; Chl b: chlorophyll b), photosynthetic parameters (P_n: net photosynthesis rate; g_s: stomatal conductance; C_i: intercellular CO_2 concentration; T_r: transpiration rate), chlorophyll fluorescence parameters (F_v/F_m: the maximum quantum efficiency of PSII photochemistry; ΦPSII: PSII operating efficiency; qP: photochemical quenching coefficient; NPQ: non-photochemical quenching) of maize plants grown under two salinity levels and treated with DCPTA at four rates of application.

Source of variation	Chl a content ($mg \cdot g^{-1}$ FW)	Chl b content ($mg \cdot g^{-1}$ FW)	P_n ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	g_s ($mmol \cdot m^{-2} \cdot s^{-1}$)	T_r ($mmol \cdot m^{-2} \cdot s^{-1}$)	C_i ($\mu mol \cdot mol^{-1}$)	F_V/F_m	ΦPSII	qP	NPQ
NaCl (mM) (N)										
0	2.93a	0.80a	15.05a	0.08a	4.67a	224.69b	0.83a	0.74a	0.64a	0.57b
150	1.47b	0.37b	9.11b	0.05b	2.30b	337.81a	0.58b	0.39b	0.40b	0.81a
DCPTA (µM) (D)										
0	2.00	0.51	11.00	0.06	3.13	298.90	0.67	0.52	0.48	0.75
20	2.29	0.60	12.30	0.07	3.62	283.03	0.69	0.56	0.54	0.68
100	2.42	0.67	13.50	0.07	3.87	251.35	0.74	0.60	0.54	0.65
400	2.09	0.56	11.52	0.06	3.32	291.72	0.72	0.58	0.50	0.68
Significance										
NaCl (mM) (N)	***	***	***	***	***	***	***	***	***	***
DCPTA (µM) (D)	***	***	***	***	***	***	***	***	***	**
N×D	**	**	**	ns	ns	ns	**	**	**	*

ns, *, **, and ***: Not significant or significant at P≤0.05, 0.01, and 0.001, respectively. The different letters within each column indicate significant differences according to Duncan's multiple-range test (P=0.05).

Table 3 Analysis of variance and mean comparisons for ion concentrations (K^+ and Na^+) and the expression of four genes (*ZmSOS1*, *ZmHKT1*, *ZmNHX1*, and *ZmSKOR*) responsible for Na^+ and/or K^+ uptake, transport and compartmentalization in the shoots and roots of maize plants grown under two salinity levels and treated with DCPTA at four rates of application.

Source of variation	Shoot							Root				
	Na^+ content ($mg \cdot g^{-1}$ DW)	K^+ content ($mg \cdot g^{-1}$ DW)	Na^+/K^+ ratio	Relative expression (RQ)				Na^+ content ($mg \cdot g^{-1}$ DW)	K^+ content ($mg \cdot g^{-1}$ DW)	Na^+/K^+ ratio	Relative expressio	
				ZmSOS1	*ZmNHX1*	*ZmHKT1*	*ZmSKOR*				*ZmSOS1*	*ZmNH*
NaCl (mM)												
0	2.43b	50.39a	21.11a	1.94b	2.10b	1.62b	1.61	5.13b	22.57a	0.23b	1.70b	1.17b
150	17.54a	33.05b	2.11b	4.72a	6.20a	3.27a	1.51	29.20a	10.64b	3.27a	2.88a	2.35a
DCPTA (µM)												
0	14.02	38.57	11.37	2.81	2.47b	1.33b	0.95b	20.37	14.27	2.79	1.77b	1.69
20	8.22	43.34	13.21	3.78	4.67a	2.73a	1.82a	16.34	18.78	1.15	2.58a	1.77
100	7.65	46.11	12.00	3.41	5.31a	3.28a	1.91a	13.64	18.91	0.86	2.53a	1.82
400	10.04	38.86	9.86	-	-	-	-	18.30	14.47	2.19	-	-
Significance												
NaCl (mM) (N)	***	***	***	***	***	***	ns	***	***	***	***	***
DCPTA (µM) (D)	***	***	**	***	***	***	***	***	***	***	***	***
N×D	***	***	ns	**	***	***	ns	***	***	***	*	ns

ns, *, **, and ***: Not significant or significant at P≤0.05, 0.01, and 0.001, respectively. The different letters within each column indicate significant differences according to Duncan's multiple-range test (P=0.05).

Figures

Figure 1

Effects of DCPTA on the growth performance of non-stressed or salinity-stressed maize plants. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl.

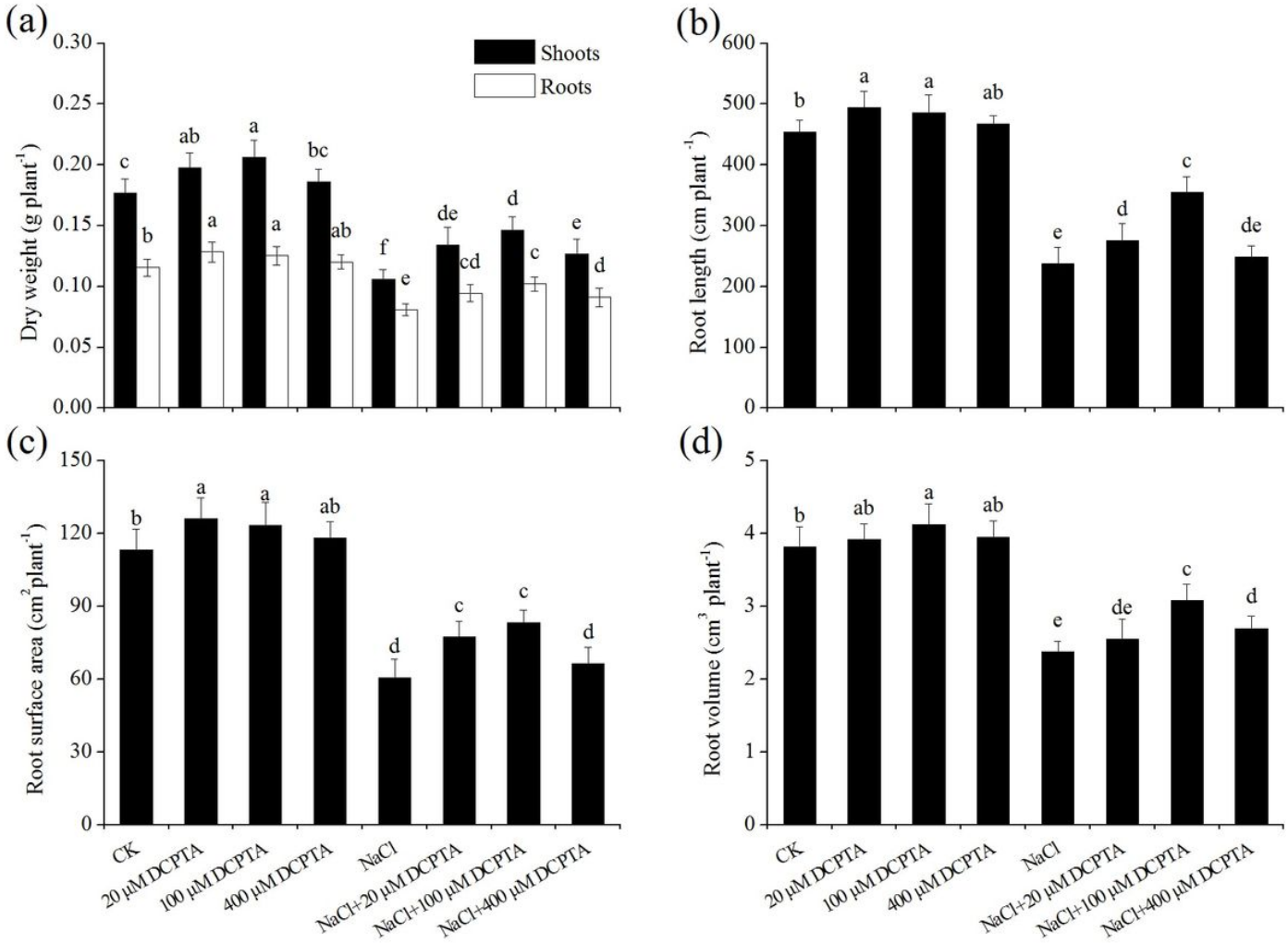

Figure 2

Effects of DCPTA on the DWs of shoot and roots (a), root length (b), root surface area (c), and root volume (d) of non-stressed and salinity-stressed maize plants. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

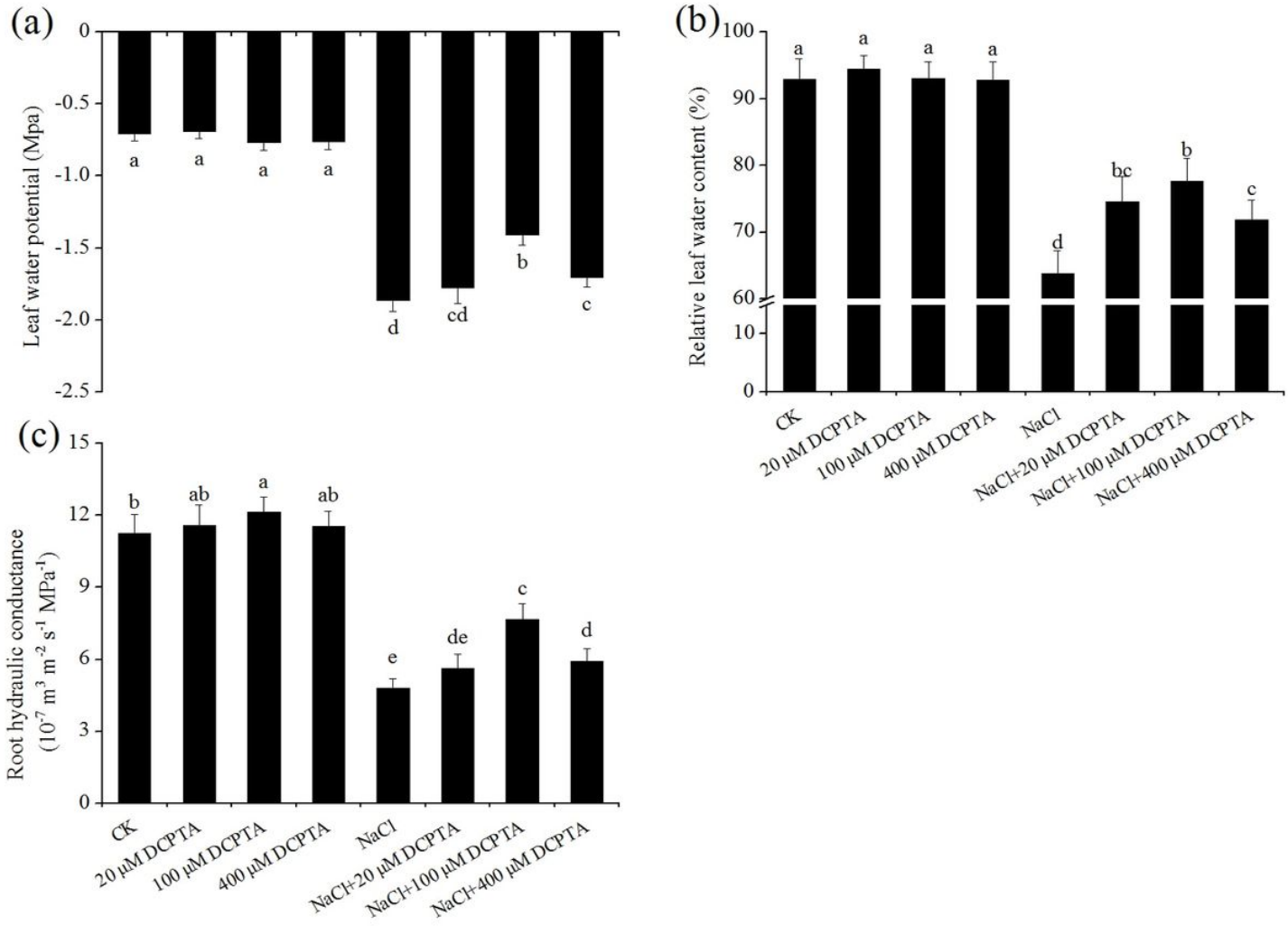

Figure 3

Effects of DCPTA on the leaf Ψω (a), LRWC (b), and root Lp (c) of non-stressed and salinity-stressed maize plants. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

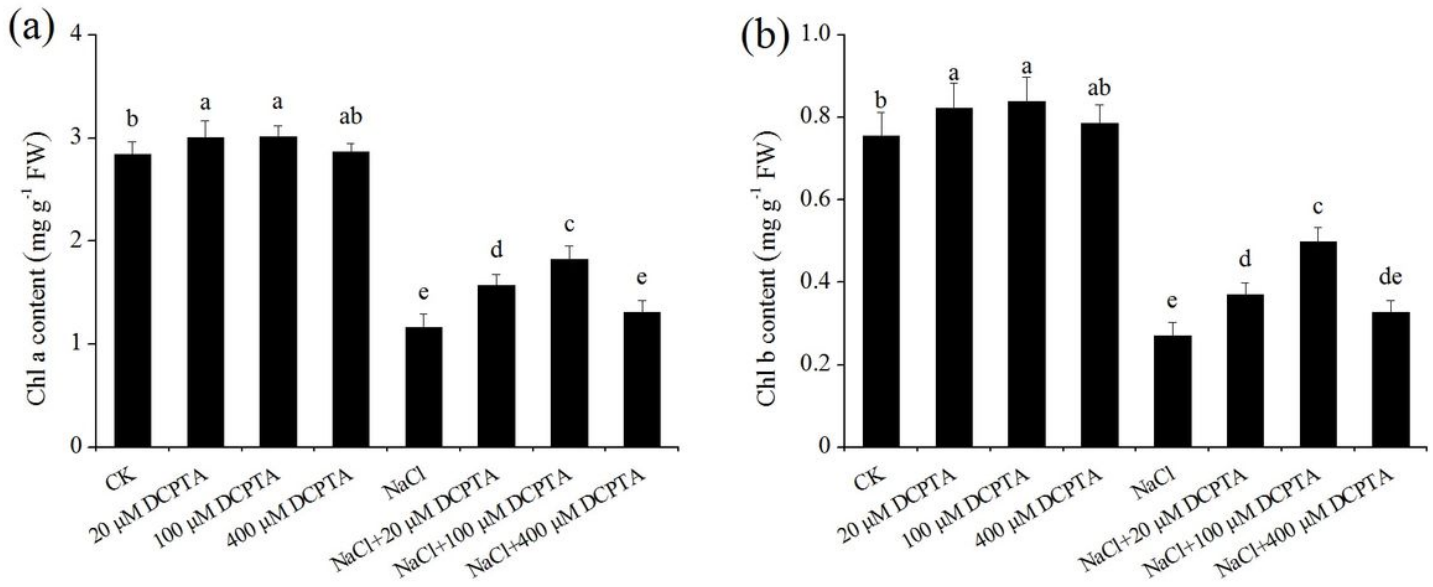

Figure 4

Effects of DCPTA on the Chl a content (a) and Chl b content (b) in the leaves of non-stressed and salinity-stressed maize seedlings. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

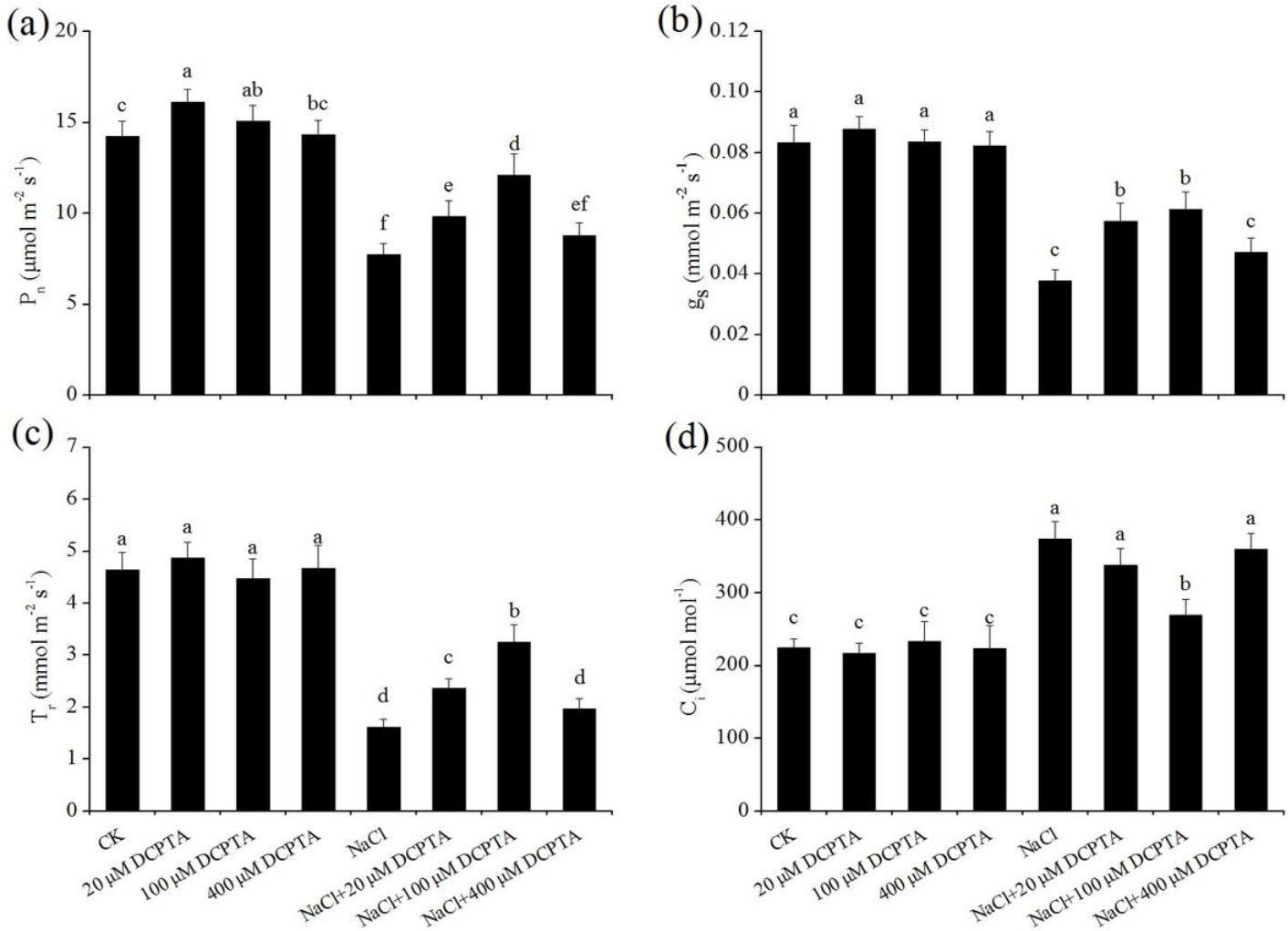

Figure 5

Effects of DCPTA on the Pn (a), gs (b), Ci (c), and Tr (d) of the leaves of non-stressed and salinity-stressed maize seedlings. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

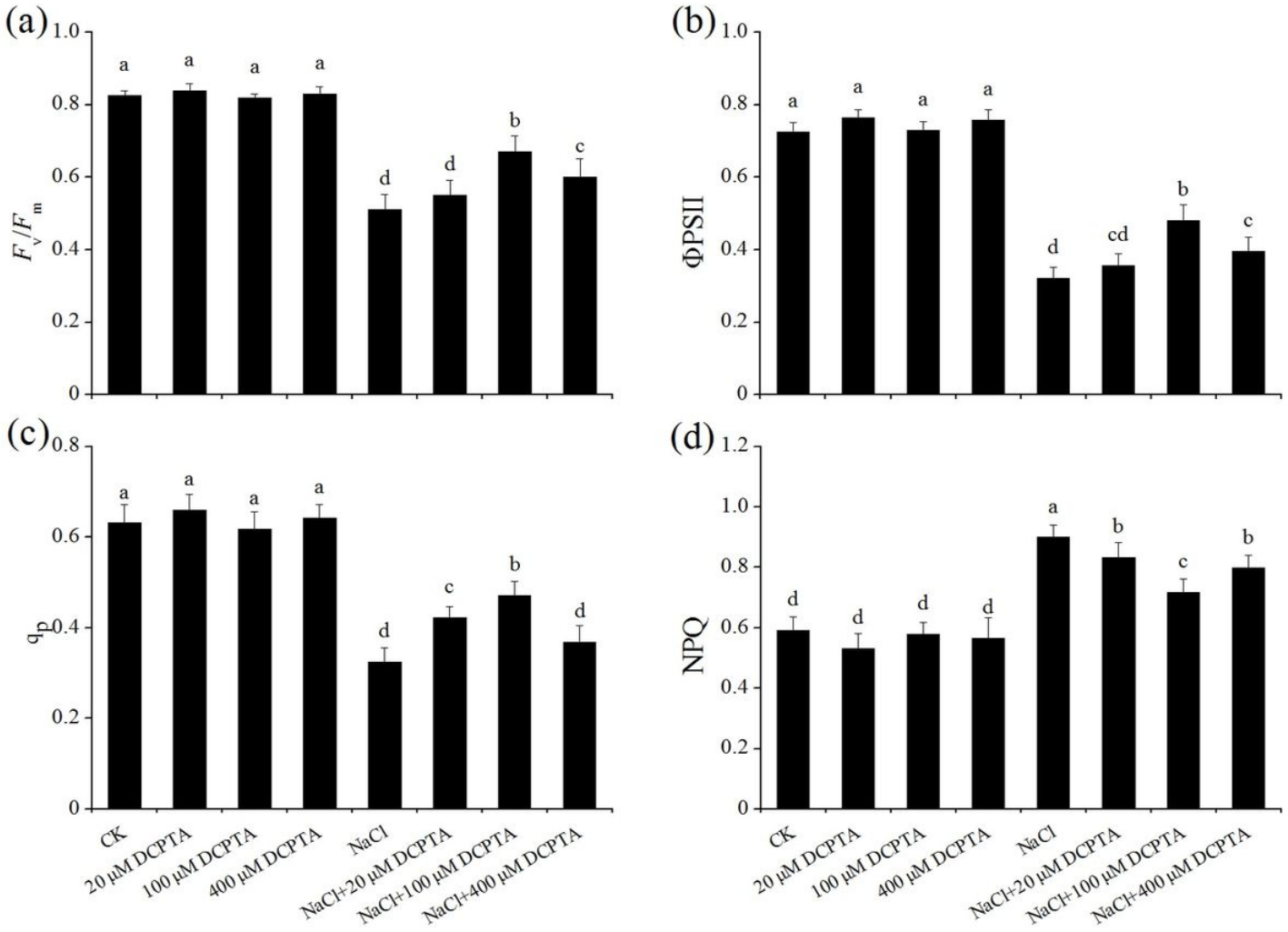

Figure 6

Effects of DCPTA on the Fv/Fm (a), ΦPSII (b), qP (c), and NPQ (d) of the leaves of non-stressed and salinity-stressed maize seedlings. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

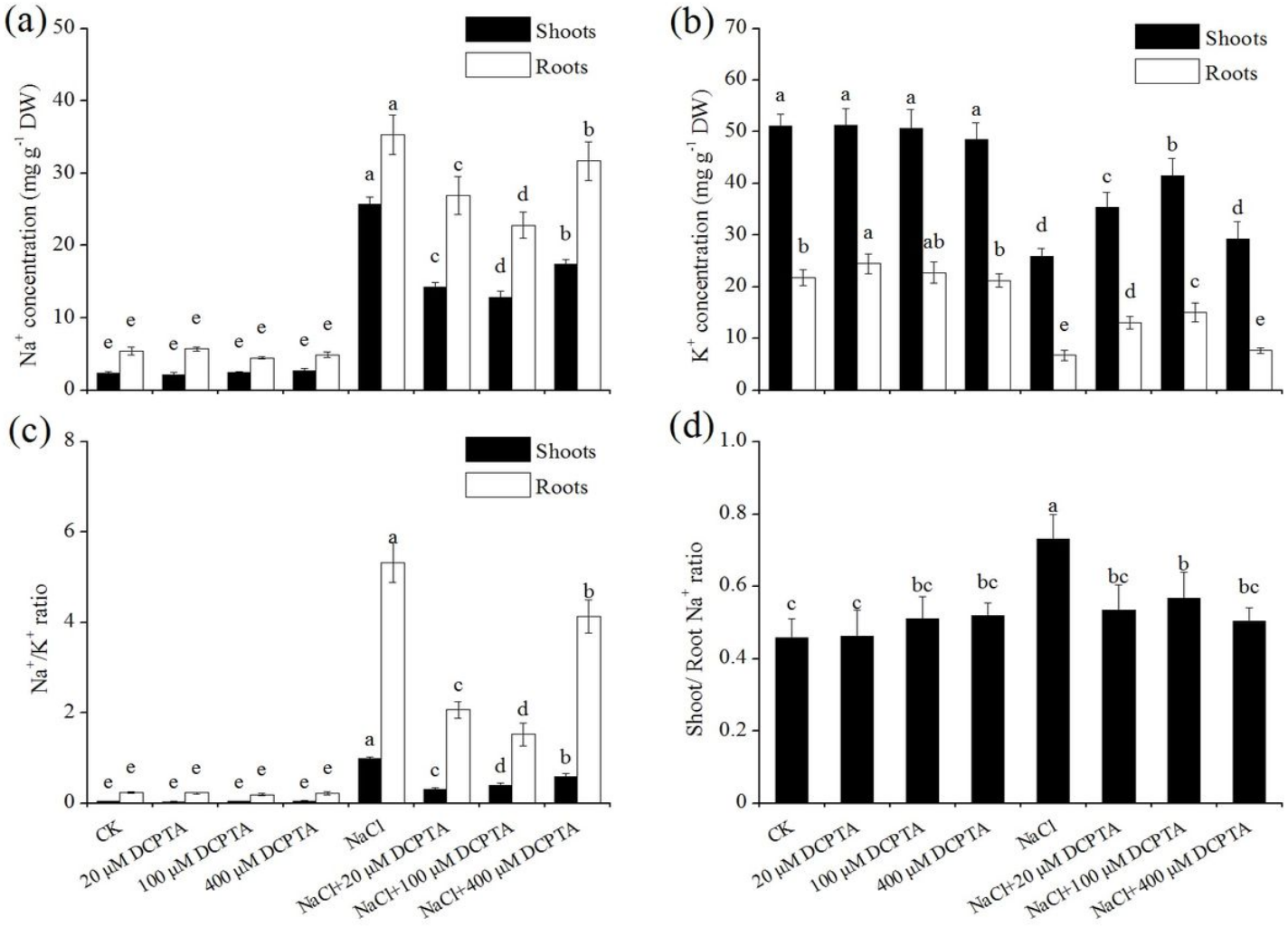

Figure 7

Effects of DCPTA on the concentrations of Na+ (a) and K+ (b) and on the Na+/K+ ratio (c) in the leaves and roots and the shoot/root Na+ ratio (d) in non-stressed and salinity-stressed maize seedlings. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

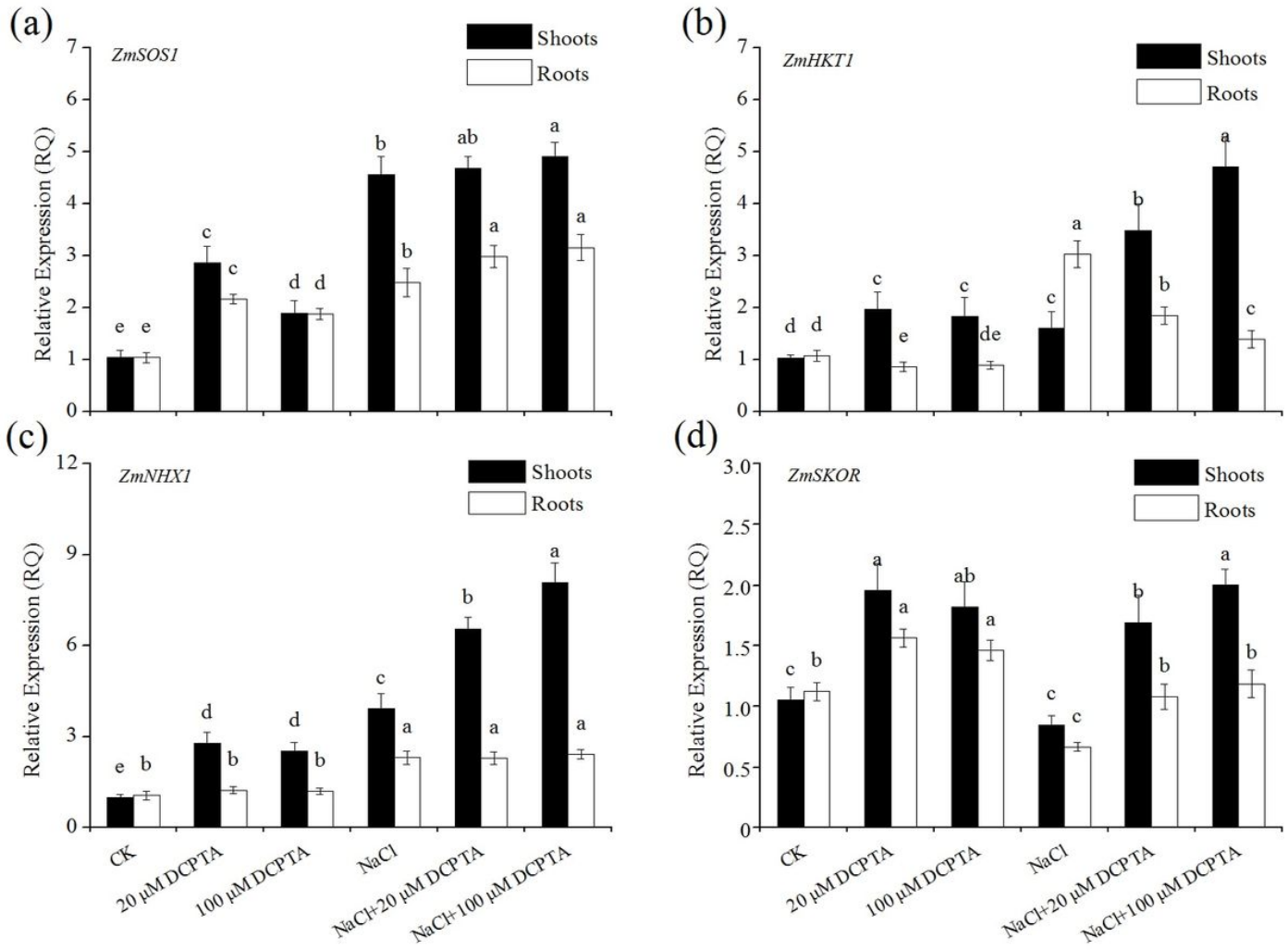

Figure 8

Effects of DCPTA on the expression of ZmSOS1 (a), ZmHKT1 (b), ZmNHX1 (c), and ZmSKOR (d) in the roots and leaves of non-stressed and salinity-stressed maize seedlings. The plants were grown in a hydroponic solution that contained 0, 20, 100, or 400 μM DCPTA with or without 150 mM NaCl. The data are the means ± SEs (n=5). The different letters on the bars indicate significant differences according to Duncan's test (P=0.05).

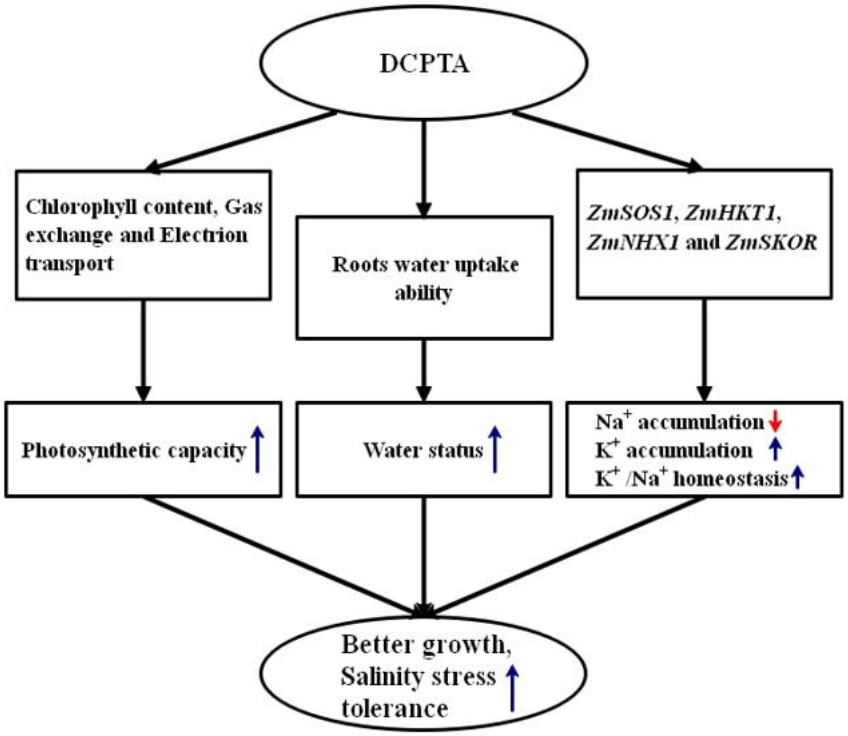

Figure 9

Schematic representation of the positive role of DCPTA on the salinity tolerance of maize. A model was developed to show that the photosynthetic capacity, water status, accumulation of Na+ and K+ and the Na+/K+ ratio were regulated by DCPTA in maize under salinity stress. The blue arrows (↑) and the red arrows (↓) represent the positive and passive roles of DCPTA, respectively.