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Research Article

Seed priming with different agents mitigate alkalinity induced oxidative damage and improves maize growth

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Abstract

Soil alkalinity is a severe threat to crop production globally as it markedly retards plant growth. Different techniques are used to mitigate alkaline stress, but priming techniques are considered the most appropriate. The current study was carried out in complete randomized design (CRD) to evaluate the effect of different priming techniques on maize crop grown under different levels of alkalinity stress. The experiment was comprised of different treatments of alkalinity stress (AS) including, control, 6 dS m^{-1} and 12 dS m^{-1} and different priming techniques including control, hydro-priming (HP), osmo-priming (OP) with potassium nitrate: KNO_3) and redox-priming (RP) with hydrogen peroxide (H_2O_2). Results indicated that alkalinity stress significantly reduced plant growth and biomass production and induced severe alterations in physiological attributes and antioxidant activities. Soil alkalinity significantly reduced the root and shoot growth and subsequent biomass production by increasing electrolyte leakage (70.60%), hydrogen peroxide (H_2O_2: 31.65%), malondialdehyde (MDA: 46.23%) and sodium (Na^+) accumulation (22.76%) and reduction in photosynthetic pigments, relative water contents (RWC), total soluble proteins (TSP) and free amino acids, potassium (K^+) accumulation. However, priming treatments significantly alleviated the alkalinity-induced toxic effects and improved plant growth. OP (KNO_3) remained the top performing. It appreciably improved plant growth owing to the improved synthesis of photosynthetic pigments, better RWC (16.42%), TSP (138.28%), FAA (178.37%), and K^+ accumulation (31.385) and improved antioxidant activities (APX and CAT) by favoring the Na^+ exclusion and maintenance of optimum Na^+/K^+. In conclusion, KNO_3 priming is an imperative seed priming practice to improve maize growth and biomass production under alkalinity stress.

Keywords: alkalinity stress; antioxidant activities; growth; ionic homeostasis; photosynthetic pigments

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Introduction

Soil salinity and alkalinity have become a severe problem across the globe, negatively affecting crop productivity (Shabala, 2013). Globally more than 1125 million hectares are affected by soil salinity and alkalization, and there are no effective measures to control this spreading (Hossain *et al.*, 2019). Soil alkalinity (SA) is characterized by higher pH (8.5-11) and higher salinity which natively affect crop growth and subsequent productivity (Amini *et al.*, 2016; Fang *et al.*, 2021). SA is induced by different salts ($NaHCO_3$ and Na_2CO_3), which disturb cell stability and destroys membrane stability, root activity and plant photosynthetic functioning (Zhang *et al.*, 2017; Kaiwen *et al.*, 2020). SA, in combination with soil salinity, disturbs soil ionic balance and reduces plant osmotic adjustment capability antioxidant activities and subsequent plant growth (Amirinejad e*t al.*, 2017; Chen *et al.*, 2017; Wang *et al.*, 2020). Moreover, SA also disturbs plant nitrogen metabolism and increases the accumulation of carbohydrates, MDA and ROS. Moreover, SA also disturbs plant nitrogen metabolism and increases the accumulation of carbohydrates, MDA and ROS due to substantial reduction in anti-oxidant activity (Ye *et al.,* 2021). Additionally, SA also reduces the synthesis of chlorophyll and destroys the structure and functioning, leading to a severe reduction in photosynthetic efficiency under SA (Li *et al.*, 2015; Xiang *et al.*, 2016).

Soil salinity and alkalinity severely affect the ions' uptake and induce an increase in accumulation of Na^+ and reduction in K^+ (Sultan *et al.*, 2021). The increase in Na^+ accumulation under SA disturbs plant osmotic balance, causes leaf senescence reduces photosynthetic pigments, and significantly reduces, reduces photosynthetic pigments, and significantly reduces plant growth (Bazzaz and Hossain, 2015; Seleiman *et al.*, 2021). Excessive Na+ accumulation under SA also causes membrane damage, resulting in a substantial increase in MDA accumulation and lipid peroxidation (Zhang and Mu, 2009; Lu *et al.*, 2009). Moreover, SA also induced a significant increase in ROS in plants (Trchounian *et al.*, 2016) which caused damages to plant proteins, lipids and DNA (Mehmood *et al.*, 2021). However, plants activate an excellent antioxidant defense system to cope with the damaging effects of these ROS (Hassan *et al.*, 2017; Aamer *et al.*, 2018; Hassan *et al.*, 2019; Hassan *et al.*, 2020; Hassan *et al.*, 2021; Imran *et al.*, 2021).

Different strategies are used across the globe to improve plant growth and productivity under soil salinity and SA. The development of tolerant cultivars is an imperative approach however it is time taking and costly process (Batool *et al.*, 2022). In this context, agronomic practices offer a quick solution to this problem. Among agronomic strategies, seed priming is an effective and economical approach to improving seed germination, seedling growth and plant metabolic activities under stress conditions (Jiménez-arias *et al.*, 2015; Migahid *et al.*, 2019). Seed priming (SP) also improves protein synthesis and anti-oxidant activities, ensuring better germination and seedling growth under stress conditions (Feghhenabi *et al.*, 2020). The increase in antioxidant activities with SP reduces ROS, improving membrane stability and decreases lipid peroxidation (Alasvandyar *et al.*, 2017; Khan *et al.*, 2019). Moreover, SP improves nutrient uptake, reduces Na^+ accumulation, and regulates ion homeostasis in plants for better growth under stress conditions (Abdelhamid *et al.*, 2019). Additionally, SP also improves the photosynthetic pigments stomata conductance and maintains water potential and plant water contents, contributing to plants adaptation to stress conditions (Tabassum *et al.*, 2018; Yang *et al.*, 2018).

Maize (*Zea mays*) is an imperious cereal crop cultivated across the globe for food and feed purposes. However, the maize crop is susceptible to abiotic stresses, which can cause very severe yield reductions yield (Ahuja *et al.*, 2010; Carpici *et al.*, 2010). Many studies are available about alkaline stress on crop growth and physiological functioning. However, limited information is available about the role of different priming agents' water (H_2O), potassium nitrate (KNO_3) and hydrogen peroxide (H_2O_2) to mitigate the harmful impacts of alkalinity stress in maize crops. We hypothesized that seed priming with different agents could mitigate the adverse impacts of SA by improving antioxidant activities, photosynthetic performance and reduced accumulation of MDA and H_2O_2. Thus, this study was performed to determine the influence of diverse seed priming agents on growth, physiological attributes, and antioxidant activities of maize crop grown under alkalinity stress.

Materials and Methods

Experimental details

The present pot study was performed using maize hybrid ('MALKA-2017') as the planting material. The pots were filled with 8 kg soil, and 15 seeds were sown in plastic pots (diameter: 28 cm) filled with soil and silt with 1:1 proportion. The study was conducted in CRD with a factorial combination comprising three replications. The study comprised of different levels of alkalinity stress 0, 6 and 12dS $m^{-1,}$ which was obtained by using $NaHCO_3$ and Na_2CO_3 in 9:1 ratio and different seed priming techniques, control, hydro-priming osmo-priming and redox-priming. We collected the soil from the agronomy field with the help of spade and sieved and mixed it with silt in 1:1 proportion. The was identified as sandy loam with pH 7.84, organic matter 0.82%, available nitrogen, phosphorus and potassium, 0.04%, 6.60 ppm, and 156 ppm, respectively (Homer and Pratt, 1961). 250 g soil from the soil samples was taken, and a soil paste was prepared and allowed for hours afterward the extract of soil was obtained, and soil saturation (%) was determined with given below formula:

$$Saturation\ (\%) = \frac{Loss\ in\ soil\ weight\ on\ drying}{Weight\ of\ soil\ after\ drying} \times 100$$

The quantity of salt (NaCl) needed to attain the desired EC values as per treatments were computed with given below formula:

$$NaCl\ required\ \left(\frac{g}{kg}\right) = \frac{TSS \times 58.5 \times saturation\ (\%)}{100 \times 100}$$

The total soluble salts (TSS) were determined as EC_2-EC_1×10. Moreover, for attaining the desired EC values (Where TSS denoted total soluble salts (mEqL^{-}1) and was calculated by multiplying the EC (6 and 12 dSm^{-1}) values, the salts were added at the rates of 1.019 and 2.178 g kg^{-1} soil.

Priming protocol

Hydro priming was performed by soaking seeds in 500 ml distilled water for 8 hours, and in osmo-priming, seeds were soaked in a 2% solution of KNO_3 for 8 hours, as in redox priming, seeds were soaked in a 3% solution of H_2O_2 for 8 hours. After soaking, the seeds were allowed to sun-dry to their original weight and then sown in filled plastic pots.

Growth parameters

Five plants were selected randomly from each pot to measure the plant height; leaves/plant, shoot and root fresh samples were weighed. After that, collected samples were oven-dried (70 °C) to determine the root and shoots dry weight.

Relative water contents (RWC) and electrical conductivity (EC)

The plant RWC was determined by the methods of Barr and Weatherley (1962). We took plant leaves and weighed them; after that, leaves were dipped in water for three hours and leaves turgid weight was computed. Later on, these leaves were over dried until constant weight and dry weight of leaves was taken, and leaf RWC was calculated by given below formula:

LRWC = (FW-DW) / (TW-DW) × 100

Fresh leaves were used to determine the electrical conductivity by following the methods of Mostofa and Fujita (2013). Fresh leaves of 0.3 g were chopped into small pieces, dipped in a test tube, and added 25 ml of distal water after two hours EC1 was obtained using EC meter and 25 ml of distal water; EC1 was obtained using EC meter after two hours. Then the test tubes were left for 24 hours, these tubes were heated in the water bath for 50 minutes at 90 °C and EC_2 was obtained using the EC meter, and EC% was determined as follow:

$$EC\% = (EC1 \div EC2\ ) \times 100$$

Photosynthetic pigments

The plant photosynthetic pigments were determined by the methods of Arnon (1949). 0.5 gram of plant leave samples were taken and grinded in 80% methanol, the extract was obtained, and absorbance was recorded at 645 nm and 663 nm to determine the chlorophyll (a and b) contents. to determine carotenoid contents, the absorbance of the extract was measured at a 480 mm spectrophotometer to determine the carotenoid contents.

Oxidative markers and total soluble protein and free amino acids determination

0.25 g fresh plant sample was homogenized in the ice bath with 5ml of 0.1% (w/v) TCA (trichloroacetic acid). After that, samples were centrifuged at 10,000 rpm for 15 minutes. Then 1ml of leaf extract was taken, 1ml of KI buffer solution and 100 µl of potassium was added and allowed in room conditions for 30 minutes, and later on, absorbance was recorded at 390 nm (Rao and Sresty, 2000). Moreover, MDA accumulation was determined with Velikova *et al.* (2000) methods. About 0.5 g frozen sample was homogenized in 5 ml TCA and centrifuged for 15 minutes at 12,000 revolutions per minute (RPM). After that, the supernatant mixture was added with 5 ml of thiobarbituric acid (TBA) and heated at 100 °C for 30 min. Then it was quickly cooled at 40 °C in ice baths. After that, the supernatant value was read at 532 and 600 nm, and MDA contents were expressed in µmol/g FW. To determine soluble proteins, we took leaf samples (0.5 g) and grounded them by adding 5 ml of potassium buffer. After that, samples were centrifuged for 15 minutes at 15000 RPM and later on, absorbance was recorded at 595 nm with a spectrophotometer (Bradford, 1976). 0.5 g of maize plant sample was grinded using potassium phosphate buffer (5 ml), and the extract was obtained and centrifuged at 1000 RPM. Later on, the absorbance of the extract was recorded at 570 nm to determine the free amino acids (Moore and Stein, 1957).

Antioxidant activities

For ascorbate peroxide (APX) determination, the mixture was prepared to contain 100- µl enzymes extract, 100 µl ascorbate (7.5-mM), 100 µl H_2O_2 (300 mM), and 2.7 ml potassium and extract were taken. Absorbance was noted at 290 nm wavelength to determine APX contents. For determination of catalase (CAT) activity, 2.5 ml potassium phosphate buffer was treated with 100 µl of H_2O_2 (5.6 mM), then adding 100 µl of plant sample and absorbance was noted at 240 nm by spectrophotometer (Aebi, 1984).

Determination of ions

The plant samples were washed with de-ionized water (dH_2O) to remove any contamination. After that, samples were dried and digested by adding a mixture of acids (HCL and HNO3) in 1:2 and later on, the concentration of Na^+ and K^+ were determined by flame photometer.

Statistical analysis

The observed data were statistically analyzed using standard variance analysis techniques. The treatment means were analyzed by the least significant difference (LSD) test at 5% of the probability level (Steel *et al.*, 1997). Moreover, graphs were produced by using sigma-plot software.

Results

Different priming agents improved the plant's growth under alkaline stress

Exposure of maize plants to 6 and 12 dsm^{-1} alkalinity stress caused a marked reduction in plant height (5.53% and 26.04%), LPP (19.93% and 45.46%), SFW (12.47% and 25.38%), SDW (41.66% and 125%), RFW (24.61% and 38.70%) and RDW (4.58% and 133.33%), respectively as compared to non-stressed control plants. On the other hand, different priming agents improved the growth parameters, like H_2O, KNO_3 and H_2O_2 increased the plant height, LPP, SFW, SDW, RFW and RDW under 6 and 12 dSm^{-1} alkalinity stress as compared to control (Table 1).

Table 1. Effect of different priming agents on the growth attributes of maize crop growth under diverse levels of alkalinity stress

Alkalinity stress	Seed priming	PH (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	LPP
0	Control	23.75f	25.33ef	6.75g	5.40e	3.46i	6.50f
6 dSm^{-1}		21.73h	21.46h	4.55h	3.59f	2.40j	4.50i
12		17.73I	17.41i	3.44h	2.42f	1.50k	3.50j
0	H_2O Priming	24.66e	29.41c	10.69cd	9.36c	7.43e	7.52e
6 dSm^{-1}		22.69g	24.22fg	7.43fg	6.21de	5.46g	5.74g
12 dSm^{-1}		18.44k	22.26h	6.26g	5.35e	4.40h	4.46i
0	KNO_3 Priming	28.65a	35.53a	16.37a	15.39a	11.43a	11.30a
6 dSm^{-1}		27.73b	33.38b	12.25bc	13.32b	10.46b	9.61c
12 dSm^{-1}		20.74i	27.20d	10.20de	9.38c	8.46d	6.46f
0	H_2O_2 Priming	25.52c	30.31c	13.32b	12.52b	9.46c	10.43b
6 dSm^{-1}		25.70d	24.46de	11.31cd	10.75c	6.50f	8.75d
12 dSm^{-1}		19.69j	23.1gh	8.98ef	7.75d	3.46i	5.05h

The value is the means of three replications with different letters indicating the significant difference ($P \leq 0.05$). PH: plant height, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, LPP: leaves per plant.

Different priming techniques improved the plant RWC and reduced the EL

The imposed alkalinity stress resulted in a considerable decline in RWC and an increase in EC level in maize plants compared to control. RWC was reduced by 6.31% and 12.40%, and EC was increased by 11.17% and 20.82% at 6 and 12 dS m^{-1} of alkalinity levels, respectively, compared to control (Figure 1a). In contrast, different priming agents mitigated the alkalinity effect on RWC and EC of maize plants, but KNO_3 showed the most effective results by increasing RWC by 13.78% and 15.19%, and decreasing EC by 29.55% and 43.71% at 6 and 12 dS m^{-1} of alkalinity stresses, respectively.

RWC also increased in plants treated with H_2O priming by 8.96% and 9.23%, and with H_2O_2 priming by 4.55% and 3.48% under 6 and 12 dSm-1 alkalinity stress. H_2O priming decreased EC level by 6.76% and 4.70%, and H_2O_2 priming by 22.20% and 31.12% under 6 and 12 dSm^{-1} alkalinities exposed plants, respectively, when compared with alkaline exposed plants only. In the absence of alkalinity stress, these priming agents increased the RWC and reduced the EC level relative to control (Figure 1b).

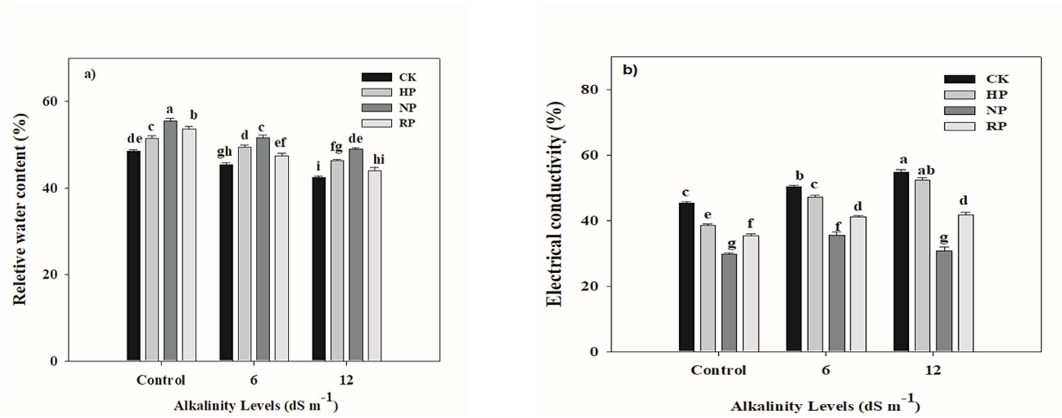

Figure 1. Effect of different seed priming agents on relative water contents (a) and electrical conductivity (b) of maize crops under diverse levels of salinity stress

CK: control, HP: hydro-priming, NP: nutrient priming, RP: redox priming. The bars are the mean value of three replications with ± SE and different letters indicating the significant difference ($P \leq 0.05$).

Different priming agents protected the photosynthetic pigments in maize leaves under alkalinity stress

Chl a, chl b and carotenoids were significantly decreased in alkalinity. Chlorophyll a content decrease by 50% and 29.54%, chl b content by 41.67% and 66.66%. Carotenoid content decreased by 6.46% and 35.48% in plants grown under 6 and 12 dS m^{-1} alkalinity stresses (Figure 2 a-c). It was worth noting that different priming agents protected photosynthetic pigments in alkalinity-stressed plants as increased in chl a content at H_2O priming (40% and 71.92%), KNO_3 priming (almost 5 and 1 time) and H_2O_2 priming (almost 3 and 7 times) under 6 and 12 dSm^{-1} alkalinity stress (Figure 2a). We observed that different priming agents also improved the level of photosynthetic pigments in non-stressed plants (Figure 2).

Different priming agents reduced the accumulation of H_2O_2 and MDA under alkalinity stress

MDA and H_2O_2 concentration considerably increased by (20.30% and 46.67%) and (92.75% and 181.69%) under 6 and 12 $dsms^{-1}$ alkalinity stressed plants, respectively, when compared with non-stressed plants (Figure 3 a, b). In contrast, their concentration declined in plants treated with priming agents compared to non-treated stressed plants. H_2O priming reduced MDA concentration by 26.33% and 48.68%, KNO_3 priming by 49.89% and 78.75%, and H_2O_2 priming by 33.59% and 56.37% and while H_2O_2 was reduced by 14.07% and 9.47% in response to H_2O priming, 5.68% and 31.91% in response of KNO_3 priming and, 32.33% and 19.44% at 6 and 12dS m^{-1} alkalinity stress, respectively (Figure 3b).

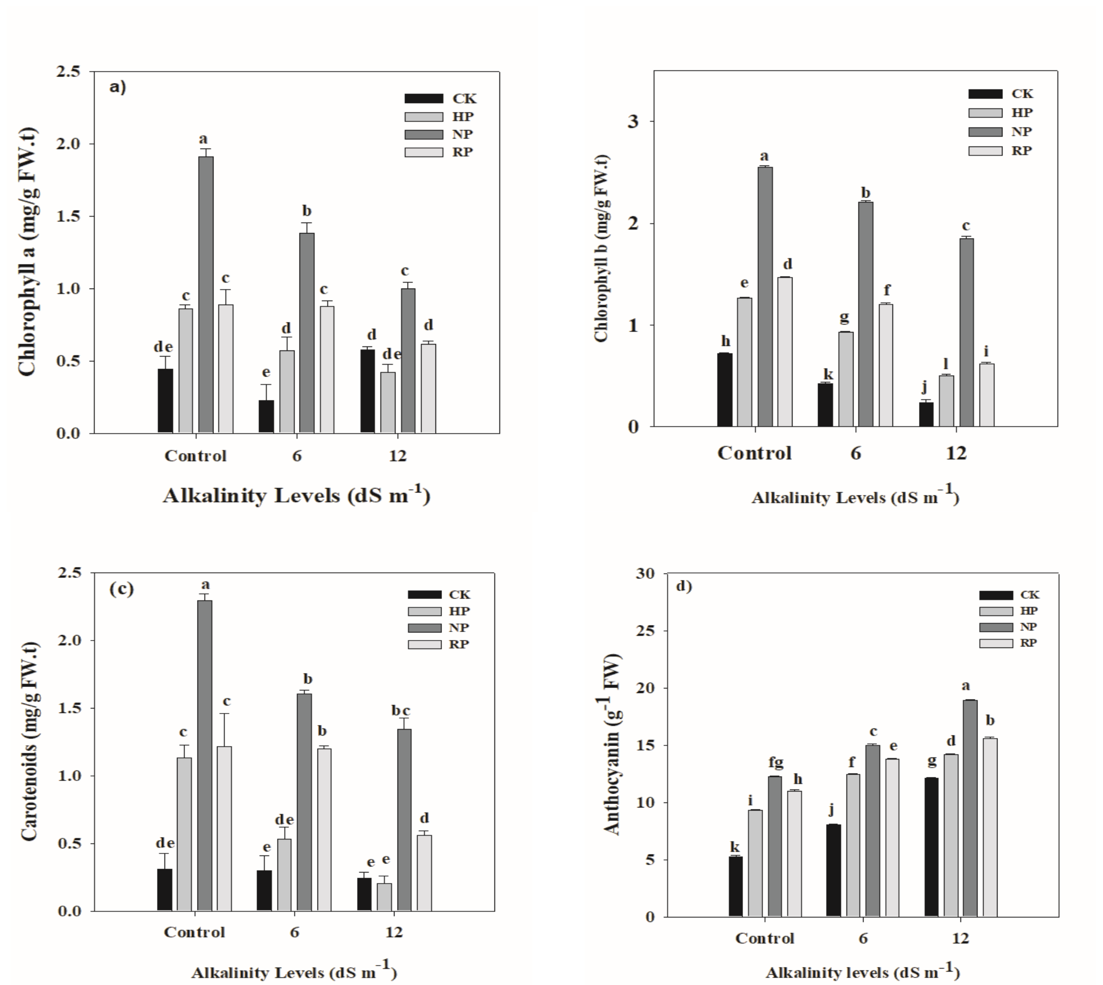

Figure 2. Effect of different seed priming agents on chlorophyll contents (a), chlorophyll b contents (b) carotenoids (c) and anthocyanin contents (d) of maize crop under diverse levels of salinity stress
CK: control, HP: hydro-priming, NP: nutrient priming, RP: redox priming. The bars are the mean value of three replications with ± SE and different letters indicating the significant difference ($P \leq 0.05$).

Different priming agents increased the total soluble proteins (TSP) and total free amino acid (TFA) in alkalinity exposed plants

There was a considerable reduction in TSP and TFA compared with alkaline free control. Total soluble proteins decreased by 12.38% and 55.40%, and total free amino acids by 25.91% and 47.05% in 6 and 12 dS m^{-1} alkalinity exposed plants, respectively, compared with stress-free plants (Figure 3 c, d). H_2O priming improved TSP (by 44.21% and 36.36%), TFA (by 48.92% and 65.51%), second priming agent KNO_3 alleviated stress by improving TSP accumulation (by 4 and 3 times) and TFA by (3 and 2 times) and third H_2O_2 treatment enhanced TSP (by almost 1.5 and 2.5 time) and TFA by (almost 1.5 and 2 times) under 6 and 12 dS m-1 alkalinity induced plants, respectively when compared with alkaline exposed plants only (Figure 3d).

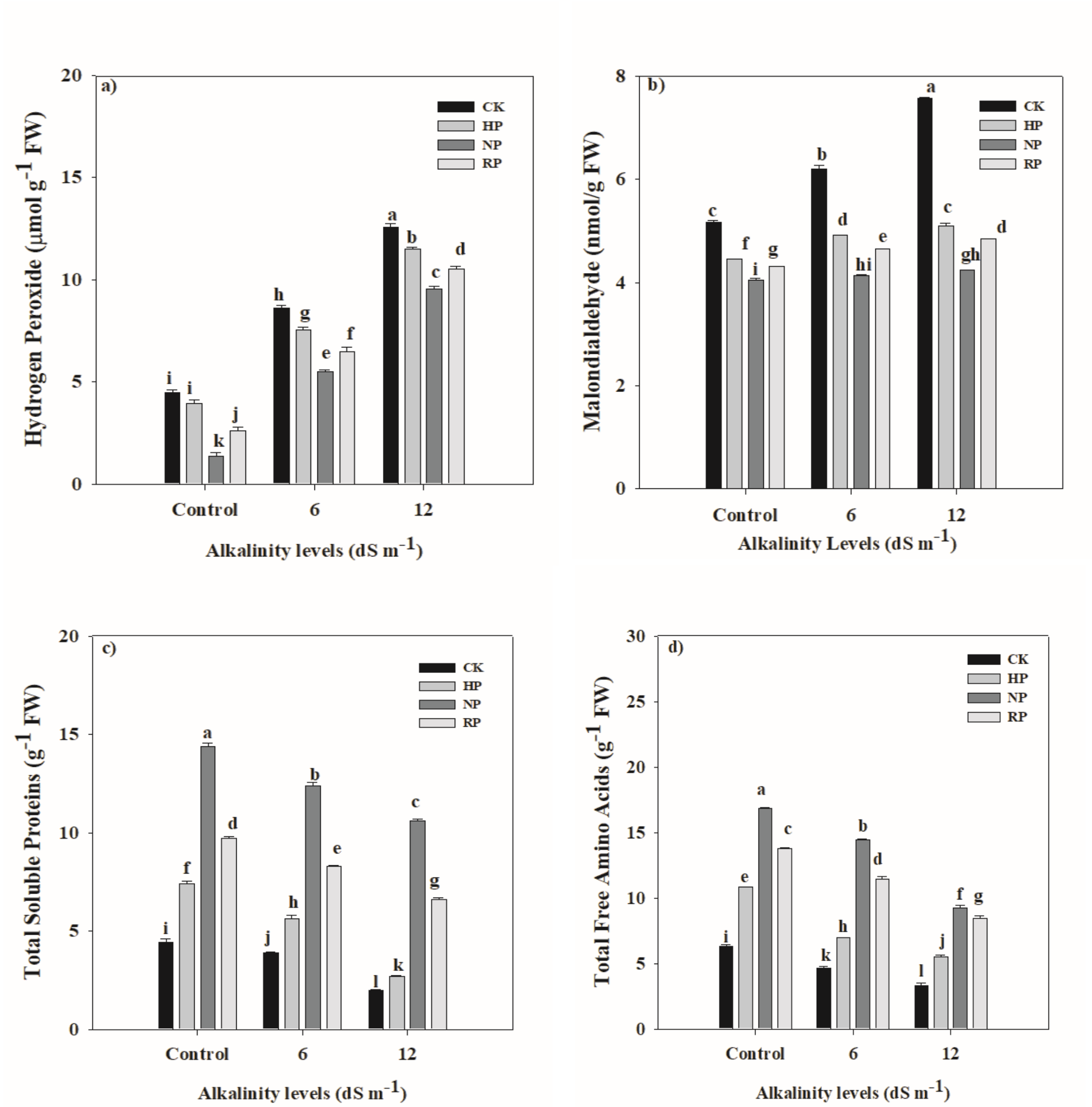

Figure 3. Effect of different seed priming agents on hydrogen peroxide (a), malondialdehyde (b) total soluble proteins (c) and free amino acid contents (d) of maize crop under diverse levels of salinity stress
CK: control, HP: hydro-priming, NP: nutrient priming, RP: redox priming. The bars are the mean value of three replications with ± SE and different letters indicating the significant difference ($P \leq 0.05$).

Different priming agents improved antioxidant activities in alkalinity stress

Changes in activities of enzymes were observed under 6 and 12 dSm^{-1} alkalinity stress. There was an increase in CAT activity by 2.5 times and APX activity by 25.51% and 87.75% at 6 and 12 dSm-1 alkalinity stress, respectively, in maize plants compared to control (Figure 4 a, b). Different priming treatments further enhanced enzymatic activities. CAT activity was increased by 57.38% and 23.89% with H_2O priming, 48.54% at KNO_3 priming 92.21% and 38.94% at H_2O_2 priming. APX activity was increased by 88.28% and 33.33% by H_2O at 6, and 12 dSm^{-1} alkalinity exposed plants, respectively (Figure 4b).

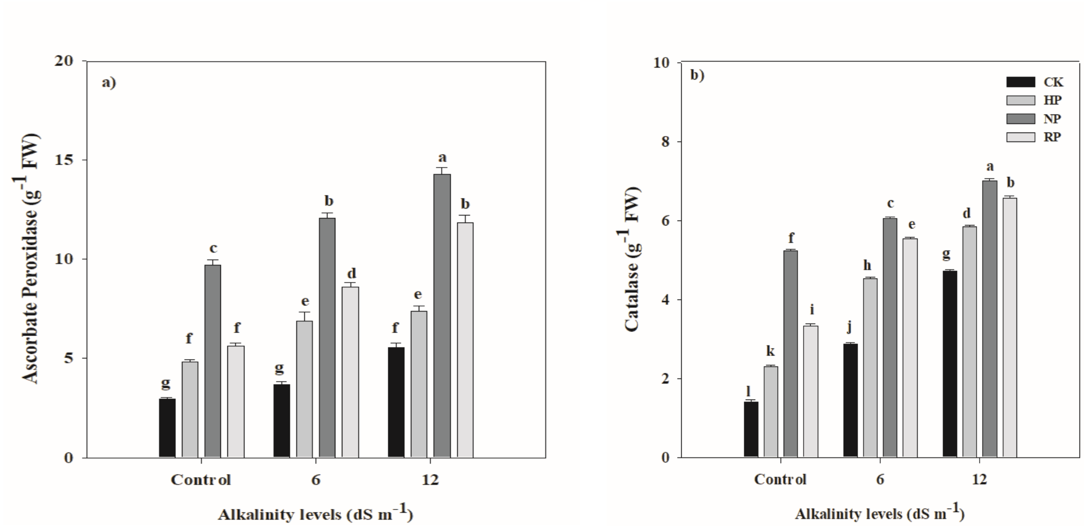

Figure 4. Effect of different seed priming agents on ascorbate peroxidase (a) and catalase (b) activity of maize crop under diverse levels of salinity stress

CK: control, HP: hydro-priming, NP: nutrient priming, RP: redox priming. The bars are mean value of three replications with ± SE and different letters indicating the significant difference ($P \leq 0.05$).

Different priming agents maintain optimum K^+ accumulation and Na^+/K^+ ratio under alkalinity stress

Mineral's analysis showed that an increased alkalinity level enhanced the Na^+/K^+ ratio compared to u control. In comparison with unstressed control, 6 and 12 dS m^{-1} alkalinity stress increased the Na^+/K^+ ratio in maize plants by 60.97% and 101.94%, respectively (Figure 5c). But different priming treatments reduced the Na^+/K^+ level by 29.16% and 32.25% at H_2O priming, by 41.66% and 45.16% at KNO_3 priming and by 33.33% and 41.93% at H_2O_2 priming under 6 and 12 dS m^{-1} alkalinity stress (Figure 5). Moreover, salt stress also significantly increased Na^+ and decreased K^+ accumulation. However, seed priming with different agents reduced the Na^+ and increased the K^+ accumulation and in this perspective KNO_3 priming remained the top performer (Figure 5).

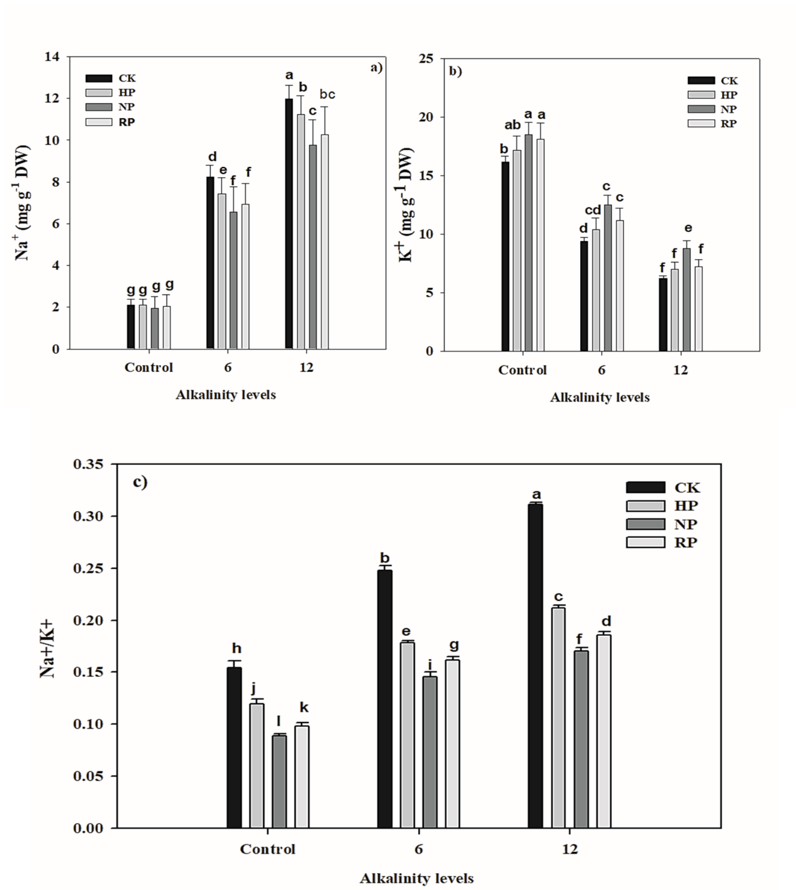

Figure 5. Effect of different seed priming agents on sodium (a) potassium (b) and Na/K ratio (c) of maize crop under diverse levels of salinity stress

CK: control, HP: hydro-priming, NP: nutrient priming, RP: redox priming. The bars are mean value of three replications with ± SE and different letters indicating the significant difference ($P \leq 0.05$).

Discussion

In the present study, we demonstrated the efficacy of different priming agents for improving the alkalinity stress tolerance hybrid maize. Soil alkalinity induced a significant reduction in maize growth and biomass production (Table 1). The reduction in maize crop growth under SA was due an increase in soil pH, which negatively affects plant growth. The higher soil pH damages the root cells and inhibits the plant growth, and leads to plant wilting and, subsequently plant death (Guo *et al.*, 2014; Wei *et al.*, 2015). Soil alkalinity also inhibited the plant height (Table 1) owing to an increase in Na^+ and reduction in K^+ accumulation in plant cells. The increase in Na+ accumulation decreased cell osmotic pressure and cell expression pressure thus, plants cannot reach to maximum size under stress conditions (Guo *et al.*, 2014). Alkalinity stress also leading to poor water and nutrient uptake and resulting in a significant reduction in growth and biomass production (Amini *et al.*, 2016). Seed priming effectively mitigated the adverse effect of SA and improved the growth and biomass production; however, seed priming with KNO_3 remained the top performer (Table 1). This increase in growth following nutrient priming can be attributed to improved root growth and subsequently improved nutrient and water uptake (Zhu *et al.*, 2011; Dai *et al.*, 2017).

The nutrient priming also improves the stomata movements and reduces the transpiration losses (Habibi, 2015; Karmollachaab and Gharineh, 2015). This results in the production of taller plants with more, with more plants biomass production (Table 1). Soil alkalinity stress significantly reduced photosynthetic pigments (Figure 2). A reduction in the synthesis of photosynthetic pigments caused a decline in food production for plant growth (Roychoudhury and Basu, 2008). Alkalinity stress increases the activity of chlorophyll degrading enzymes which are a primary reason for reducing chlorophyll contents under SA (Rao and Rao, 2013). Moreover, SA also decreases Mg^{2+} uptake, which is the building block of chlorophyll. The reduction in Mg^{2+} uptake also leads to chlorophyll degradation under SA (Shi, 1997). However, seed priming appreciable alleviated the effects of SA and improved the photosynthetic pigments (Figure 2). Seed priming maintains the membrane integrity and antioxidant activity and restricting the activities of enzymes involved in chlorophyll degradation (Dai *et al.*, 2017), thus resulting in better chlorophyll contents under SA (Figure 2). Moreover, SA also causes a reduction in carotenoid contents (Figure 2) which works as scavenging of free radicals provoked by ROS (Gururani *et al.*, 2015). Seed priming with KNO_3 effectively increased the carotenoid contents in maize plants (Figure 2) which could improve the capacity of this compound to diminish the damage caused by ROS under SA.

Excessive accumulation of ROS by SA causes oxidative damage in plants. SA caused a significant increase in MDA and H_2O_2 accumulation (Figure 3). Soil alkalinity stress induces acid-base disturbance in plant cells and increases H_2O_2 accumulation (Gill and Tuteja, 2010). Moreover, SA also increased the MDA accumulation (Figure 3) due to a reduction in membrane stability and an increase in specific ion toxicity (Zhanwu *et al.*, 2014). During SA, excessive Na^+ participates in ROS production by working as signaling molecular in signal transduction pathways and excessive ROS production cause cell damage and even cell death (Kariola *et al.*, 2005). The antioxidant enzymes (APX and CAT) activities increased under SA, which was further enhanced by different seed priming agents (Figure 4). The seed priming appreciably improved the antioxidant activities by increasing the expression of stress-responsive genes and resulting in a significant increase in SA tolerance (Abdel-Latef and Tran, 2016). Soil alkalinity induced a significant reduction in accumulation of TSP and FAA; however, seed priming significantly increased the accumulation of both TSP and FAA (Figure 3). The increase in TSP and FAA with seed priming can be attributed to the fact: seed priming improved the mRNA functioning and formation of DNA, which favors a substantial increase in TSP and FAA accumulation (Abdel-Latef and Tran, 2016).

The higher K^+ and lower Na^+ ions in plant cytoplasm are essential for normal enzymatic functioning in plant cells. In the current study, SA significantly increased the Na^+ ions accumulation while decreasing the K^+ ion accumulation (Figure 5). The excessive accumulation of Na^+ in plant cells causes ionic toxicity because Na^+ is very toxic for plants for plant growth (James *et al.*, 2006; Chen *et al.*, 2012). Plants have a Na^+/H^+ antiport responsible for Na^+ exclusion and entrance of K^+ ions into plant cells (Zhu, 2003). However, in salinity and alkalinity stress, due to lower external proton concentration, the exchange capacity of Na^+/K^+ anti-porters are significantly reduced, leading to the reduction in exclusion of Na^+ and resulting in significant Na^+ ions accumulation in plant cells (Zhu, 2003; Munns and Tester, 2008). Thus, reducing the Na^+ exclusion might be the reason of the increase in Na^+ accumulation in maize plants (Figure 5). Osmotic stress is not considered to be the cause of increased Na^+ in plant cells under SA. At the same time, higher pH is also a significant cause of specific ion toxicity. Higher soil pH owing to SA decreases the ability of plants to absorb Na^+ and K^+ ions that disturb the balance between Na^+ and K^+ and (Shi, 2005; Zhang and Mu, 2009) and resulting in a reduction in K^+ ions uptake and accumulation (Zhang and Mu, 2009). Seed priming significantly reduced the Na^+ accumulation and increased the K^+ accumulation, thus maintaining higher K^+/Na^+ ration (Figure 5). The seed priming with different agents reduced the apoplastic Na^+ absorption by plant roots and resulted in a reduction in Na^+ accumulation under SA (Wang *et al.*, 2015). Moreover, increase in K^+ uptake following KNO_3 priming could be its promotive affect on plasma membrane H^+-ATPases (Karmollachaab and Gharineh, 2015). The lower Na^+/K^+ ratio is a good sign of balancing the impacts of seed priming on K^+ and Na^+ uptake under SA, encouraging seed priming to improve crop performance under SA stress.

Conclusions

Soil alkalinity induced a significant reduction in growth and biomass production of maize owing to disrupted ionic balance, increased MDA and H_2O_2 accumulation and reduced synthesis of photosynthetic pigments soluble proteins, free amino and plant water contents. Seed priming markedly reduced the harmful impacts of alkalinity stress. However, osmo-priming gave the better performance associated with higher antioxidant activities, photosynthetic pigments, soluble protein and free amino acids and reduced MDA and H_2O_2 accumulation. Therefore, it is recommended that osmo-priming can be used as essential priming practices to improve crop productivity under alkalinity stress. However, future studies are needed to optimize the recommended dose for these priming agents for different crops under diverse climate conditions before recommending it for the farming community.

Authors' Contributions

Conceptualization; I.K. and M.U.C., Data collection: H.Z., Writing-original draft; I.K., M.U.C., M.U.H., and H.Z., Writing-reviewing editing; A.M., R.M., F.A., M.A.A., F.B., F.M., and S.H.Q.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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