Received: 25 August 2020 | Revised: 10 December 2020 | Accepted: 14 December 2020

DOI: 10.1111/jac.12477

DROUGHT STRESS

Journal of Agronomy and Crop Science WILEY

Potassium, an important element to improve water use efficiency and growth parameters in quinoa (*Chenopodium quinoa*) under saline conditions

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Abstract

Due to changes in the ecosystem and misuse of resources, salinity also increases. Approximately 20% of all irrigated land is affected by salinity and this will increase over time. Therefore, it is necessary to develop more environmentally friendly agricultural techniques but also to exploit potential crops with high nutritional value and tolerance to salinity like quinoa (*Chenopodium quinoa* Willd.). In this context, potassium is an essential macronutrient for plant growth and development. Furthermore, one of the strategies of some salt-tolerant plants is to increase the uptake of potassium under saline conditions such that the K^+/Na^+ ratio is maintained for a proper osmotic regulation in cells. Therefore, a study was conducted to investigate the effect of different concentrations of potassium (0.5, 2 and 6 mM K) on quinoa under different salinities (0, 100 and 200 mM NaCl). According to the results, an adequate supply of potassium under moderate salinity conditions benefited the plant growth, with a higher potassium uptake in the presence of salt. Under saline conditions, plant transpiration decreased significantly with a high correlation with stomatal density and a greater water use efficiency. Therefore, under saline conditions, adequate doses of potassium are highly recommended in quinoa cultivation.

KEYWORDS

elemental analysis, enzyme activity, gas exchange, salt stress, stomatal density, water use efficiency

1 | INTRODUCTION

Over time, the world population is growing rapidly and will increase from 7.7 billion to 9.7 billion by 2050 (United Nations–UN, 2019). To be able to provide the growing population with sufficient food, a significant increase in plant production must be achieved. This should meet the food needs of future generations without depleting the planet's ecological and energy resources. However, the production of agricultural products is limited by numerous biotic (e.g. insects, pathogens and weeds) and abiotic factors (e.g. drought, salinity, frost and waterlogging), which reduce the quality and quantity of plant products (Wang et al., 2013). Currently, salt is degrading 20% of the world's irrigated land and causing around US$27.3 billion per year in economic losses, according to the assessment from the United Nations University (Smith, 2014). Salt stress leads to nutrient disturbances in plants, where the transport and availability of nutrients are severely impaired. The nutrient deficiencies and imbalance in saline environments are mainly

caused by the excess of Na^+ and Cl^- ions which compete with other ions like K^+, Ca^{2+}, Mg^{2+} and NO_3^-, restricting plant growth. Plant growth is restricted by osmotic and toxic effects of ions, resulting in reduced nutrient uptake and translocation, especially K^+ (Wang et al., 2013). Two successive reactions occur in plants under salt stress. First, there is a rapid osmotic shock in which the availability of water is reduced. The leaf relative water content, leaf water potential, stomatal conductance, solute potential and turgor potential are decreased (Ahmad et al., 2013), thus reducing plant growth. This is followed by an ion homeostasis phase (Orcutt & Nilsen, 2000) in which salt toxicity is triggered and the senescence of mature leaves is accelerated, also known as second phase. To avoid this second phase, plants have several mechanisms including the restriction of Na^+ uptake and exclusion, cellular compartmentalization of Na^+ in the vacuole, antioxidant regulation, compatible solutes (osmolytes), morphological adaptations (e.g. salt bladders), among others. The mechanism used depends on the group of plants, glycophytes or halophytes, and on each species as well.

Halophytes, which are salt-tolerant plant species, are of particular importance as they can remove excess of salt from the soil and water and at the same time food products can be obtained. In the course of their evolution, different physiological mechanisms have been developed to cope with numerous stress conditions (Wang et al., 2013). Thus, quinoa (*Chenopodium quinoa* Willd.) is considered a very valuable crop species, as it is particularly tolerant to abiotic stress factors such as drought, frost (Jancurová et al., 2009) and salinity (Adolf et al., 2013). Quinoa is a plant species which belongs to the family Amaranthaceae which originates from the Andes region in South America (Fernando et al., 2000) and has been cultivated there for 7,000 years (Adolf et al., 2013; Haros & Schoenlechner, 2017). It also has many nutritious properties, such as high protein content in the seeds (8%–22%), which is higher than in cereals such as barley, rice and wheat, which makes it suitable for both human consumption (Jancurová et al., 2009) and animal products (Bhargava et al., 2006). The seeds, which are gluten-free, are composed of essential amino acids, vitamins (A, B_2, E) and important minerals; therefore, they are considered a valuable plant product for human nutrition. As an important seed plant, it is becoming increasingly important also for other regions (Adolf et al., 2013). In addition to its tolerance to frost (Jancurová et al., 2009), it can also be grown well in dry and saline soils (Adolf et al., 2013), with salt concentrations up to 500 mM NaCl (Turcios et al., 2016), which are salinity levels as high as those present in sea water, making it an attractive plant in arid and semi-arid regions where water scarcity and salinity are major problems (Fernando et al., 2000). Due to its salt tolerance, quinoa can thus produce protein-rich grains even under conditions that are extreme for regular crop plant species (James, 2009), but some nutrients like K could enhance its productivity under stress conditions.

Potassium is an important nutrient for all plants which constitutes 1%–10% of the dry matter (Wakeel, 2013), varying depending on the plant species, plant organs and nutrition, and it is therefore one of the most abundant mineral nutrients. When K^+ is available to plants, it can be taken up by passive (diffusion and mass flow mainly) and active (high and low affinity K^+ transporters) transport systems. Then K^+ is transported symplastically to the xylem from all regions of the root. The delivery of K^+ within the shoot is largely determined by transpirational water flows, and the shoot apoplastic K^+ concentration at the point of xylem unloading approximates 5–20 mM, which enables expanding shoot cells to acquire K^+ through plasma membrane cation channels (Karley & White, 2009). Its cellular levels are significant (typically around 100 mM) and are highly regulated (Ahmad & Maathuis, 2014), but can also vary depending on its availability. Its deficiency can inhibit the photosynthetic activity and trigger the formation of reactive oxygen species – ROS (Tränkner et al., 2018), which leads to considerable cell damage. It is particularly important to ensure plant growth even under abiotic and biotic stress. Potassium is an essential nutrient for enzyme activity, energy transfer, protein synthesis, osmoregulation, stoma movement, phloem transport, anion-cation balance and stress resistance (Wang et al., 2013). To achieve good photosynthetic performance under different stress factors, optimal conditions must be created under a sufficient nutrient supply. Thus, sufficient K^+ and Mg^{2+} must be available for the photosynthesis and transport of assimilates (Tränkner et al., 2018). Potassium is particularly important as an inorganic osmotic element in plant cells, and thus, sufficient K^+ supply is crucial for the regulation of turgor-driven processes like stomatal movement and cell elongation (Tränkner et al., 2018). Cellular K^+ retention and cytosolic K^+ homeostasis are essential for salinity tolerance, which also depends on a good interaction of export and import of K^+ at the tonoplast and plasma membrane. Potassium storage is crucial for quinoa and its salinity tolerance, as it can store high K^+ concentrations in the cotyledons already in the seedling stage, and in the xylem and leaf juice during the continuous growth phase (Adolf et al., 2013). Under saline conditions, Na^+ is also found at high concentrations, where the K^+ availability is necessary for maintaining the K^+/Na^+ ratio in plants. Thus, an increased Na^+ uptake also requires an increased amount of K^+ to be transported to the shoot (Adolf et al., 2013). Thus, for an optimal plant growth, the Na^+ uptake must be reduced and the K^+ uptake increased (Wakeel, 2013), since a higher K^+/Na^+ ratio improves the tolerance of the plant (Hu & Schmidhalter, 2005). However, K^+ and Na^+ have similar physicochemical properties, which means that they compete for important binding sites, including low affinity (e.g. non-selective cation channels – NSCC) and high affinity (e.g. K^+ UPtake – KUP – and high affinity K^+ transporter – HKT) transporters (Wang et al., 2013).

It is particularly important to ensure plant growth even under abiotic and biotic stress, whereby K^+ is considered an essential nutrient being shown to be relevant in stress tolerance. The aim of this work is to investigate the response of quinoa to salinity using different K concentrations in the culture medium on the basis of different morphological and physiological parameters. To gain an insight into the importance of K for the prevention of salt stress in quinoa, an experiment under controlled conditions in hydroponics was carried out. For this, two factors were used: different K concentrations (0.5, 2 and 6 mM K) and different salt concentrations (0, 100 and 200 mM NaCl). According to the results, it

was observed that the tolerance against salt stress increases with increasing K doses in the culture medium.

2 | MATERIALS AND METHODS

2.1 | Experiment set-up

Seeds of *C. quinoa* var. Titicaca were obtained from the Institute of Botany, Leibniz University Hannover, but originating in Peru, close to Lake Titicaca. Seeds were germinated in filter paper rolls with a nutrient solution consisting of 1 mM $CaSO_4$ for 9 days with a temperature of around 24°C. Then the plants were transferred to the different experimental units, under hydroponic conditions. Each experimental unit consisted of a pot with a capacity of 6 L. This was filled with 5 L of nutrient solution consisting of different NaCl and K concentrations as well as other essential nutrients: 3 mM NH_4NO_3, 1 mM $MgSO_4$*7 H_2O, 1 mM $CaCl_2$*2 H_2O, 0.25 mM $Ca(H_2PO_4)_2$*H_2O, 0.1 mM Fe-EDTA, 25 µM H_3BO_3, 2 µM $ZnSO_4$*7 H_2O, 2 µM $MnSO_4$*H_2O, 0.5 µM $CuSO_4$*5 H_2O, 0.075 µM $H_{24}Mo_7N_6O_{24}$*4 H_2O. In addition to these nutrients, three different K concentrations (0.5, 2 and 6 mM K) supplied as KCl were combined with three different salt concentrations (0, 100 and 200 mM NaCl) making a total of nine different treatments. The different K concentrations were chosen based on previous experiments where 0.2 mM K showed clearly K deficiencies, and with lower K concentrations plants showed severe symptoms and did not grow (Figure 1S). Three replicates per treatment were used, with a total of 27 experimental units with one plant in each. Each experimental unit was supplied with oxygen into the nutrient solution to support root respiration. In this experiment, physiological parameters like biomass production, elemental analysis, Na/K ratio, photosynthesis rate, water use efficiency, leaf area, among others were analysed, as detailed below.

The plants first were cultivated in the nutrient solution without salt. Then the salt was added stepwise 11 days later, after the plants were adapted to the corresponding nutrient solution. For the nine experimental units with a salt content of 200 mM, the total amount was divided so that first 100 mM was added to the plant's nutrient solution and 2 days later the plant received the other 100 mM NaCl. The nutrient solution of the plants was changed every 7 days after an acclimatization period of 2 weeks. Furthermore, the plants were watered regularly to maintain the water level and therefore the nutrient and salt concentration. The plants were grown in a climate chamber (16 hr light at 25°C/8 hr dark at 18°C, 60% humidity) at the Institute of Applied Plant Nutrition (IAPN), University of Göttingen, Germany.

2.2 | Plant growth and leaf gas exchange

The aboveground plant height and total plant biomass were determined on the day of harvest, which took place 7 weeks after sowing. Shortly before the end of the experiment, the gas exchange was measured on a representative leaf of each plant. Net assimilation rates (A), transpiration rates and stomatal conductance were determined on the youngest fully expanded leaves (GFS-3000, Heinz Walz GmbH). Cuvette conditions were set as follows: 22°C, 55% rel. humidity, 400 ppm CO_2, photosynthetic photon flux density of 1,000 µmol m^{-2} s^{-1} generated by blue and red LEDs. After reaching stable values due to leaf adjustment to cuvette conditions, fluxes were averaged over 5 min.

2.3 | Leaf stomatal density

To determine the stomatal density, a leaf imprint using polysiloxane precision impression material (Xantopren L blue, Heraeus Kulzer) was taken on young fully expanded leaves of each plant before harvesting (7 weeks after sowing). Imprints were taken on the abaxial leaf surface by applying a thin layer of the impression material quickly on a surface of about 1 cm^2 in the middle of the leaf blade. Leaves of same age were taken in all plants. After the imprints were dried, they were carefully detached from the leaves and the stomatal density was determined under a light microscope (Axio, Carl Zeiss Microscopy) using a micrometre. The number of stomata per square millimetre at three different imprint locations was counted, and then the average was calculated.

2.4 | Plant harvest and leaf area

Seven weeks after sowing, the plants were harvested and the root and shoot fresh mass was determined in each plant. The total leaf area of each plant was measured by a leaf area meter (LI-3100C Area Meter, Li-Cor Biosciences). Each leaf was scanned individually by the instrument. The harvested plant material was finally dried in a drying cabinet at 60°C for 6 days to remove the remaining moisture from the plants, and the dry mass (DM) was recorded. Leaf-area ratio (LAR) was obtained by dividing the leaf area by total DM. Dry plant material was subsequently ground into fine powder for elemental analysis.

2.5 | Biomass water use efficiency – WUE

To measure the transpiration of the plants, the weight of each experimental unit was recorded before and after each change of nutrient solution. This was also necessary once the plants were watered. Thus, it was possible to continuously document the plant transpiration. Despite the fact that the pots were closed to avoid the loss of water due to evaporation, other containers with the same solution but without plants were used to verify that evaporation could be excluded. Total transpiration was obtained by summing up water consumption by the plants over the entire growth period. Biomass-WUE was calculated as the ratio of total plant DM to total water consumption per pot.

2.6 | Elemental analysis

For the analysis of K and Na concentrations in the plant material, different parts of the plant for each treatment were taken including roots, a mixture of leaves and a mixture of the aboveground part (shoots). For this, 100 mg of dried plant material was weighed and transferred to a digestion beaker. After complete transfer of the samples into the digestion beakers, 4 ml of 65% (v/v) nitric acid (HNO_3) and 2 ml of 30% (v/v) hydrogen peroxide (H_2O_2) were added. The digestion beakers were closed and placed in the microwave (Ethos.lab, MLS) for 120 min at 200°C and 15 bar. A certified reference material (apple leaf, SRM 1515, National Institute of Standards and Technology) was included in each batch of microwave digestion. After a short cooling down period under the fume hood, the vessels were opened, allowing nitrous gases to escape. In the next step, the samples were transferred into a 25-ml volumetric flask and filled up to the calibration mark with ultrapure water (Seralpur). These samples were finally placed in falcon tubes for the analysis. Samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP 6000 ICP Spectrometer, Thermo Fisher Scientific Corporation). To ensure accuracy of measurements, a sample of the certified reference material was included after approx. each 20 samples.

2.7 | Determination of hydrogen peroxidase and enzyme activity

To analyse the stress level in plants associated with salinity and K deficiency, the enzymatic activity of catalase (CAT) and ascorbate peroxidase (APX) was measured, as well as the concentration of H_2O_2 in the plant material. For this, fresh leaf material was taken from the plant before harvest and immediately frozen in liquid nitrogen to stop the enzymatic activity. For ROS scavenging enzyme activities, 0.5 g of leaf sample was harvested and immediately frozen in liquid nitrogen until extraction. All extraction steps were performed at 4°C. An extraction buffer (KH_xPO_4) was prepared by titrating 50 mM KH_2PO_4 (acid buffer, pH ~ 4.5) to 50 mM K_2HPO_4 (base buffer, pH ~ 9.2) until reaching pH ~ 7.6, and including 1% (w/v) polyvinylpyrrolidone (PVP) and 0.1 mM ethylenediaminetetraacetate (EDTA). The leaf samples were homogenized in 5 ml of the extraction buffer, and samples were centrifuged at 16,000 g at 4°C for 20 min. The supernatant (crude extract) was transferred to a precooled reaction vessel and stored at −20°C until measurement of CAT and APX activity.

CAT was measured according to Beers and Sizer (1952). For CAT activity assay, the 0.3 ml reaction mixture contained 50 mM KH_xPO_4 buffer with 0.1 mM EDTA, 10 mM H_2O_2 and 10 µl of crude extract. The reaction was initiated with 100 mM H_2O_2, and the reduction of H_2O_2 concentration was measured spectrometrically (EPOCH, BioTec, USA/8453 UV-VIS Spectroscopy System, Agilent) following the decrease in absorbance at 230 nm.

For APX activity assay, the 0.3 ml reaction mixture contained 0.5 mM ascorbic acid, 50 mM phosphate buffer, 1 mM EDTA, 0.5 mM H_2O_2 and 10 µl of plant extract. The reaction was assayed spectrometrically (EPOCH, BioTec, USA/8453 UV-VIS Spectroscopy System, Agilent). The reaction was initiated by adding 10 µl of 15 mM of H_2O_2, and APX activity was assayed spectrometrically following the decrease in absorbance at 290 nm (Nakano & Asada, 1981).

The concentration of H_2O_2 was determined using ferrous ammonium sulphate xylenol orange (FOX) solution as described by Wolff (1994) and modified by Cheeseman (2009). Briefly, 3 leaf discs were cut from fully expanded young leaves using a cork borer (0.46 cm^2) and were transferred to 1 ml acidified acetone (acidified with 25 mM H_2SO_4). Samples were immediately frozen in liquid nitrogen. FOX solution containing 250 µM ferrous ammonium sulphate, 100 mM sorbitol, 100 µM xylenol orange and 25 mM H_2SO_4 was prepared prior to thawing the samples at room temperature for 45 min. Then, 50 µl of each sample or standard was added to 1 ml of FOX solution. Samples and standards were incubated at room temperature for another 45 min. Shortly before the end of this time, 300 µl of each sample or standard was pipetted in duplicates into a 96-well microtitre plate. The concentration of H_2O_2 was quantified spectrometrically (EPOCH, BioTec, USA/8453 UV-VIS Spectroscopy System, Agilent) at 550 nm and subtracting the background at 850 nm using a standard curve ranging from 0 to 100 µM.

2.8 | Statistical analysis

All statistical analyses were conducted using R, version 3.1.1 (R Core Team, 2014) and InfoStat software, version 2016e (Di Rienzo et al., 2016). The effects and interaction of the two main factors: K and NaCl, on the different parameters were analysed through a two-way analysis of variance (ANOVA). The Tukey multiple comparison test with a significance level of 0.05 was done to determine which means differ from the rest. Principal component analysis (PCA) was done using InfoStat. PCA was performed to determine which variables are associated with the different NaCl and K levels. For this, two principal components (PC1 and PC2) were used to explain the most variance of the data.

3 | RESULTS AND DISCUSSION

3.1 | K and Na nutritional status

To investigate the response of quinoa to salinity under different K concentrations, different tissue K and Na concentrations were sought to be induced by supplying different K and NaCl concentrations in the growth medium. As expected, decreasing K supply decreased tissue K concentration, and increasing NaCl supply increased Na concentrations (Table 1). Under non-saline conditions, K concentrations in shoots decreased from 57.07 mg/g DM under 6 mM K supply to 31.7 mg/g DM and 28.12 mg/g DM under 2 mM K and 0.5 mM K supply, respectively. It is important to highlight that under saline conditions (200 mM NaCl) K concentration in

TABLE 1 Element concentration (mg/g DM) in roots, leaves and shoots of quinoa cultivated under different NaCl and K concentrations

K (mM)	NaCl (mM)	K (mg g^{-1} DM)	Na (mg g^{-1} DM)
Roots			
0.5	0	13.61 ± 0.42cd	9.64 ± 0.79e
2		16.52 ± 0.98cd	11.55 ± 1.05e
6		37.88 ± 0.68b	0.79 ± 0.12e
0.5	100	8.73 ± 0.76d	61.86 ± 1.65bc
2		18.87 ± 1.65c	51.4 ± 0.74c
6		38.93 ± 1.19b	31.44 ± 2.06d
0.5	200	10.15 ± 0.63cd	83.42 ± 4.81a
2		17.97 ± 0.57cd	76.04 ± 2.05ab
6		50.49 ± 5.14a	55.68 ± 6.73c
Leaves			
0.5	0	16.64 ± 0.71d	1.5 ± 0.15e
2		51.91 ± 4.71bc	0.9 ± 0.09e
6		76.22 ± 2.46a	0.38 ± 0.02e
0.5	100	13.89 ± 0.85d	51.76 ± 1.9bc
2		39.01 ± 0.64c	38.16 ± 1.71d
6		55.2 ± 5.11b	36.86 ± 1.32d
0.5	200	17.78 ± 2.04d	89.37 ± 1.81a
2		46.16 ± 2.25bc	62.04 ± 6.38b
6		77.61 ± 2.39a	45.68 ± 2.11cd
Shoots			
0.5	0	28.12 ± 4bc	1.79 ± 0.14e
2		31.7 ± 2.67bc	1.59 ± 0.49e
6		57.07 ± 1.55b	0.69 ± 0.33e
0.5	100	13.71 ± 3c	58.04 ± 2.87b
2		36.51 ± 8.78bc	29.73 ± 0.35d
6		51.38 ± 4.85b	29.26 ± 1.46d
0.5	200	28.19 ± 7.37bc	73.7 ± 1.47a
2		59.55 ± 5.17b	44.43 ± 3.85c
6		97.73 ± 16.29a	29.96 ± 1.23d

Note: Values represent mean ± *SE* values of three biological replicates per treatment. Significant differences ($p \leq .05$) between treatments are indicated by different letters. The analysis of variance was carried out independently for roots, leaves and shoots.

shoots was higher compared to the control (non-saline conditions; Table 1). Critical tissue K concentrations are not yet defined for quinoa, and concentrations which fall into a deficiency range are not yet quantified. However, the K concentrations under lowest K supply are below typical ranges reported for many crops (Bergmann, 1988). Spinach (*Spinacia oleracea* L.) and sugar beet (*Beta vulgaris* L.) belong to the order of the Caryophyllales, family of Amaranthaceae like quinoa; hence, their nutrient requirements might reflect those for quinoa the best. For sugar beet, a typical nutrient concentration in mature leaves is described to be 20–60 mg/g DM (Bryson et al., 2014) and for spinach 35–53 mg/g DM (Bergmann, 1988). Cole et al. (2020) reported K concentrations between 53 and 35 mg/g in control quinoa plants supplied with 4 mM K, and between 25 and 17 mg/g for quinoa plants supplied with 0.4 mM K, depending on the growth stage. Thus, we conclude that the K concentrations under low K supply in our study fall into a deficient range which is also indicated by reduced growth performance (Table 2).

Under low K^+ availability in the nutrient solution (<1 mM K), K^+ is actively taken up by plants , needing extra energy, so if there is enough Na^+ in solution, it is easier for the plant to passively take up this ion, thereby saving energy (Ragel et al., 2019; Wakeel et al., 2011). Sodium can partially replace K^+ in non-specific functions (Krishnasamy et al., 2014) like osmotic adjustment due to their similar physicochemical properties. Therefore, a lack of K^+ can be compensated by Na^+, so that the plant does not show a reduction in yield (Wakeel et al., 2010). In sugar beet, K^+ could be substituted by Na^+ to an extent of 98.75% without inhibiting growth, whereas protein synthesis appeared to be already affected when 97.50% of K^+ was substituted by Na^+ (Faust & Schubert, 2016). In contrast, in the glycophyte wheat only up to 15% K^+ in the cells is exchangeable by Na^+ (Zörb et al., 2014). For this reason, under moderate salt concentrations quinoa biomass increased (Table 2), as also described by Turcios et al. (2016). In this sense, in highly K-fixing soils, a K deficiency can be counteracted by applying Na (Wakeel et al., 2010). Likewise, an increased K^+ content can to some extent compensate for the stress induced by Na^+ (Mäser et al., 2002). This can be seen also in Table 1, where under moderate K supply (2 mM K), K increased in the plant shoots when salt concentration increased in the nutrient solution. However, under this condition, where Na^+ and K^+ are available to plants, they can also be passively taken up through non-selective channels without the use of extra energy. Therefore, as salinity increases, the K^+ content also increases in the shoots (Table 1). This is also a strategy against saline stress of some halophytes such as quinoa, where the K^+/Na^+ ratio is maintained at high levels (Iqbal et al., 2019). The regulation of Na^+ uptake and transport within the plant under salt stress has been previously investigated in the context of maintaining high tissue K^+/Na^+ ratio, and therefore high cytosolic K^+/Na^+ ratio, which has become a key of salt tolerance. Probably the most important salt stress adaptation toward sustaining enhanced cytosolic K^+/Na^+ ratio is prevention of cellular K^+ efflux (Assaha et al., 2017).

Under sufficient K supply (6 mM K), plants absorbed more K^+, specially under high salt concentration (200 mM NaCl), but plants avoided taking up Na^+, maintaining Na^+ at lower concentrations compared to other low K treatments. Plants use different ways for Na^+ exclusion such as Na^+/H^+ antiporters in root plasma membrane and encoded by salt overly sensitive (SOS1) gene (Tanveer & Shah, 2017), and HKT transporters preventing shoot Na^+ over-accumulation (Hauser & Horie, 2010). However, it is mostly carried out at the expense of energy use, reducing the biomass yield as can be seen in Table 2. Efflux of Na^+ could potentially also take place to some extent by Na-translocating ATPases; however, the thermodynamic cost of moving Na^+ from the cytosol to the soil solution by this

TABLE 2 Plant growth parameters in quinoa cultivated under different NaCl and K concentrations

K (mM)	NaCl (mM)	Shoot FM (g)	Root FM (g)	Total FM (g)	Shoot DM (g)	Root DM (g)	Total DM (g)	Shoot DM/root DM	Plant height (cm)	Leaf area (cm^2/plant)	LAR (cm^2/g DM)
0.5	0	72.34 ± 4.87e	98.2 ± 18.24bc	170.54 ± 23.1c	11.59 ± 1.29d	4.18 ± 0.55cd	15.78 ± 1.82c	2.79 ± 0.11cd	54.5 ± 3.69b	1525.25 ± 119.68d	97.96 ± 6.95b
2		157.09 ± 2.47bcd	151.36 ± 8.89a	308.45 ± 11.24ab	22.96 ± 0.88ab	8.7 ± 0.47a	31.66 ± 1.31a	2.64 ± 0.07d	70.67 ± 2.59a	3,011.74 ± 54.25abc	95.41 ± 3.69b
6		197.92 ± 4.67ab	99.62 ± 12.07bc	297.54 ± 16.74ab	27.41 ± 0.4a	7.59 ± 0.81ab	35.01 ± 1.06a	3.69 ± 0.38ab	70.17 ± 0.17a	3,591.75 ± 35.3ab	102.76 ± 2.78ab
0.5	100	125.24 ± 4.11cde	114.24 ± 2.35abc	239.48 ± 3.52bc	13.98 ± 0.5cd	4.98 ± 0.24bcd	18.96 ± 0.71bc	2.81 ± 0.09cd	49.83 ± 1.45bc	2,860.41 ± 376.1abc	152.32 ± 24.69a
2		177.15 ± 23.93abc	134.99 ± 10.34ab	312.14 ± 30.45ab	21.61 ± 3.56abc	7.28 ± 1.03ab	28.9 ± 4.56ab	2.95 ± 0.13bcd	53 ± 2.31b	3,105.62 ± 359.32abc	109.39 ± 7.44ab
6		220.6 ± 13.06a	110.1 ± 9.5abc	330.7 ± 19.31a	26.09 ± 1.79a	6.96 ± 0.53abc	33.05 ± 2.29a	3.76 ± 0.1ab	54.67 ± 2.13b	3,855.67 ± 370a	117.19 ± 10.69ab
0.5	200	119.56 ± 9.73de	71.03 ± 5.36cd	190.59 ± 14.83c	11.23 ± 1.21d	3.67 ± 0.23d	14.9 ± 1.42c	3.05 ± 0.18bcd	38.17 ± 2.17d	2,166.31 ± 145.84cd	146.51 ± 7.48ab
2		147.79 ± 2.18bcd	80.48 ± 1.97cd	228.27 ± 0.37bc	15.45 ± 0.62bcd	4.27 ± 0.09cd	19.72 ± 0.64bc	3.62 ± 0.15abc	41.5 ± 1.8cd	2,611.3 ± 61.47bcd	132.47 ± 1.24ab
6		118.72 ± 16.72de	48.38 ± 5.45d	167.1 ± 21.87c	12.85 ± 2.46d	3.07 ± 0.47d	15.91 ± 2.93c	4.14 ± 0.15a	37 ± 2.65d	2,101.52 ± 173.77cd	136.5 ± 12.21ab

Note: Values represent mean ± *SE* values of three biological replicates per treatment. Significant differences between treatments are indicated by different letters ($p \leq .05$).
Abbreviations: DM, dry mass; FM, fresh mass; LAR, leaf area ratio.

mechanism would be very high, namely 1 ATP per 1 Na^+ extruded (Edelstein et al., 2011).

To have a complete overview regarding the relationship between mineral K nutrition and plant salt tolerance, more studies are needed; particularly, both critical K concentrations and K^+/Na^+ ratios should be targeted in research in future.

3.2 | Growth parameters: fresh and dry mass, plant height and leaf-area ratio

Most growth parameters were significantly affected by salinity and K concentrations. According to the results, both factors K ($p < .0001$) and NaCl ($p = .0004$) had significant effects on shoot fresh mass. Plants grown with a K concentration of 6 and 100 mM NaCl reached an average value of 220.60 g, which showed no significant difference to the plants cultivated under 2 mM K and 100 mM NaCl with a value of 177.15 g. However, there was a highly significant difference to the concentration 0.5 mM K under non-saline conditions with an average of 72.34 g (Table 2). For the factor NaCl, the highest value was achieved by the concentration of 100 mM NaCl with an average of 174.33 g and this was significantly different from the concentrations 0 mM NaCl and 200 mM NaCl with 142.45 and 128.69 g, respectively.

The analysis of variance for DM shoots revealed highly significant differences for both factors ($p < .0001$). The highest DM was achieved by the plants grown with 6 mM K in the absence of salt with a value of 27.41 g, but it did not differ significantly with respect to 2 mM K and a salinity of 100 mM NaCl with a value of 21.61 g (Table 2). However, there was a significant difference to the plants cultivated with a concentration of 0.5 mM K and 200 mM NaCl, where only 11.23 g DM was obtained. These results are supported by preliminary experiments where K concentrations higher than 0.1 mM K significantly increased the biomass production (Figure 2S). Compared to the fresh mass, the highest value for shoot DM was not reached at 6 mM K and 100 mM NaCl, but at 6 mM K under non-saline conditions (Table 2).

Smaller ions like Na^+ have a higher surface charge; therefore, the hydration shell is larger (Wakeel et al., 2011), allowing a greater quantity of water to be adsorbed. For this reason, in the case of quinoa plants which have the ability to take up large amounts of Na^+, the water content increases in the plant tissue and leads to succulence, yielding a greater amount of fresh matter under moderate saline stress conditions (100 mM NaCl).

A significant effect of both factors (K: $p < .0001$; NaCl: $p < .0001$) on the total DM (roots + shoots) can also be observed. The highest values of total DM were obtained from the plants cultivated under a K concentration between 2 mM and 6 mM K and a NaCl concentration between 0 and 100 mM NaCl with no significant differences between each other at these concentrations (Table 2). The highest total dry matter was achieved by the plants cultivated under non-saline conditions and 6 mM K followed by 100 mM NaCl and 6 mM K with values of 35.01 and 33.05 g, respectively. The lowest value was 14.90 g, and

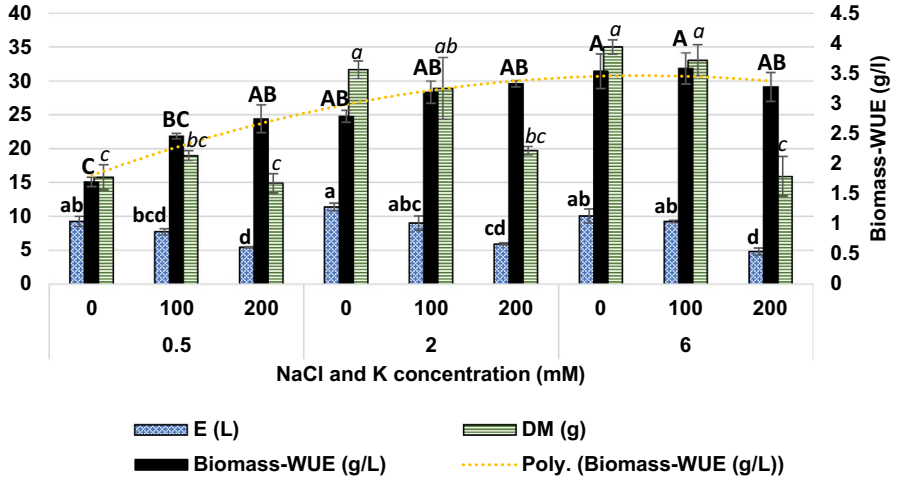

FIGURE 1 Transpiration and biomass water use efficiency (Biomass-WUE) in quinoa cultivated under different NaCl (first row in the bottom of figure) and K (second row in the bottom of figure) concentrations (mM). DM, dry mass; E, plant transpiration; Poly, polynomial trendline. Columns represent mean ± *SE* values of three biological replicates per treatment. Significant differences ($p \leq .05$) between treatments are indicated by different letters

it was obtained from the plants cultivated at 0.5 mM K and 200 mM NaCl (Table 2). According to these results, a K concentration ranging from 2 to 6 mM is important to increase the production of biomass in quinoa under non-saline and moderate saline stress conditions. In the same way, both factors K and NaCl significantly influenced the plant height reaching greater height under non-saline conditions and with K concentrations varying from 2 to 6 mM. The plant height was inhibited with increasing NaCl concentration (Table 2). Potassium had small influence on the plant height, as long as sufficient K was available, since the plants with 2 mM K and 6 mM K were about the same size, but the plants with low K concentration (0.5 mM) were significantly smaller (Table 2). This shows that the plant achieves a higher growth with sufficient K^+, but an excess of this does not cause additional plant growth. Based on these results, it is clear that the plant needs sufficient K^+, especially under salt stress to maintain the K^+/Na^+ ratio, thus ensuring the plant growth as discussed below.

Regarding shoot/root ratio, the analysis of variance shows a high significant difference for the factor K ($p < .0001$) and for NaCl ($p = .0025$). Plants grown with sufficient K produced a greater leaf biomass, so this ratio increased. A concentration of 6 mM K produced the highest values followed by 2 mM K (Table 2). In plants grown under saline conditions, the root biomass decreased, so the shoot/root ratio increased. A salt concentration of 200 mM NaCl and 6 mM K yielded the highest shoot/root ratio with a value of 4.14 followed by 100 mM NaCl and 6 mM K with 3.76 (Table 2).

Total leaf area was significantly affected by K and NaCl concentrations. The highest leaf area was determined by a K concentration of 6 mM K and 100 mM NaCl with 3,855.67 cm^2. The lowest leaf area was 1525.25 cm^2 obtained from the plants cultivated at 0.5 mM K under non-saline conditions (Table 2). Thus, an increase in K concentration is accompanied by an increase in leaf area. In the same way, plants cultivated with 100 mM NaCl reached the highest leaf area and thus the highest fresh biomass. Regarding the leaf-area ratio (LAR), there was a significant difference only for NaCl ($p = .0009$). The highest mean values were achieved under moderate saline conditions (100 mM NaCl) and 0.5 mM K with 152.32 cm^2/g DM due to the low biomass yield under these conditions (Table 2). The lowest mean value was 95.41 cm^2/g DM which was obtained under non-saline conditions and 2 mM K, as a result of an increase in the biomass production and a decrease in leaf area (Table 2). The leaves of the plants cultivated without salt were smaller compared to plants grown with salt; therefore, the lowest LAR was obtained under this condition. Since LAR measures the efficiency with which a plant deploys its photosynthetic resources, the smaller leaves of the plants cultivated under non-saline conditions are more efficient producing high amount of biomass.

Since the highest growth parameter values were achieved at 2 mM K and 6 mM K, and between 0 mM NaCl and 100 mM NaCl, the plants could tolerate 100 mM NaCl well and a rapid osmotic adjustment in the plant could be achieved by loading the xylem with Na^+ and K^+ (Shabala et al., 2013; Shabala & Pottosin, 2014). Under high salt conditions, increased Na^+ concentration have led to a significant decrease in the K^+/Na^+ ratio in the plant tissue, as a result of which the plant was able to develop less biomass (Wakeel, 2013). A certain amount of K^+ is therefore required to balance the K^+/Na^+ ratio for a good plant growth (Adolf et al., 2013). The high importance of K becomes clear when looking at the dry matter values of both, shoots and roots. At 0.5 mM K, the K deficiency clearly reflected the biomass yield. Since K^+ critically contribute to the photosynthesis process and the subsequent long-distance transport of photoassimilates (Tränkner et al., 2018), its deficiency would restrict the plant growth process. Potassium is also considered the most important inorganic osmotic compound in plant cells where its lack prevents cell elongation, which leads to smaller mesophyll cells (Tränkner et al., 2018). Under saline conditions, the disturbance of nutrient translocation from roots to shoots is often also caused by Na^+, especially in cases of K^+ deficiency (Wang et al., 2013). Other parameters such as transpiration and water use efficiency can also be affected under saline conditions as discussed below.

3.3 | Effect of salt and potassium on transpiration and biomass water use efficiency

Both factors K and NaCl had a significant effect on biomass-WUE. The highest biomass-WUE (3.58 g/L) was reached by plants grown

with 6 mM K and 100 mM NaCl, while the lowest value (1.70 g/L) was obtained from plants cultivated with 0.5 mM K and without salt (Figure 1). The biomass-WUE is influenced by dry matter and transpiration. As discussed above, the dry matter formation under 6 mM K supply was highest, resulting in a high leaf area. However, the high leaf area did not increase the transpiration. Total transpiration (E) of the plants during the growing phase was not affected by K supply ($p = .0632$). Hence, higher biomass-WUE under high K is due to higher biomass formation. In contrast, the factor NaCl had a significant effect ($p < .0001$) on E, with a total E of 10.23 L per plant under non-saline conditions, followed by an average value of 8.65 L for plants grown at 100 mM NaCl and finally 5.37 L at 200 mM NaCl (Figure 1). The NaCl concentration was decisive for E, where E decreased significantly with increasing NaCl (Figure 1). This is related to the fact that the plants suffer from salt stress, as the water uptake is restricted due to a lower water potential. Therefore, the water balance in the plant is affected, with possible toxic effects causing anatomical and physiological changes in the plant (Wang et al., 2013). As a result, the plant restricts its transpiration due to low water absorption from the rhizosphere (Adolf et al., 2013; Nultsch, 2012). Likewise, by inhibiting water uptake, the plant cannot absorb sufficient essential nutrients from the nutrient solution, which restricts its growth, regardless of its resistance status (Pitann et al., 2009). This can also be seen in Table 2, where the plants were significantly smaller when the salt concentration increased. If both salt levels are compared, 0 mM NaCl and 200 mM NaCl, the plants that were not exposed to salt were almost twice as higher as the plants with a concentration of 200 mM NaCl.

To obtain insights into the transpiration independent of the total leaf area, the stomata density was also determined. According to the analysis of variance for the variable stomatal density, there was no significant difference between the three K concentrations ($p = .0546$), but interestingly, there was a high significant influence by the different NaCl concentrations ($p < .0001$). Salinity reduced the stomatal density. The lowest value of 39.07 stomata/mm^2 was obtained at 200 mM NaCl and 6 mM K (Figure 2), whereas the highest value was obtained under non-saline conditions and 0.5 mM K, with a density of 104.11 stomata/mm^2.

According to these results, apart from the known strategies that quinoa has to tolerate salinity (e.g. accumulation of Na^+ in the roots and leaves as showed in Table 1, osmotic adjustment, ion homeostasis, increased antioxidant response, salt bladders), it is capable of drastically reducing the number of stomata per unit area to decrease transpiration. This makes water use more efficient (Figure 1) and therefore able to tolerate and grow in saline environments. This reduction in stomatal density under saline conditions has been shown by recent studies (Adolf et al., 2013) to control transpiration and water use efficiency, but this mechanism has not been further explored in quinoa. In sweet basil, salinity induced by 100 and 200 mM NaCl reduced stomatal density in a cultivar being able to adapt to salt stress, whereas stomata density in a non-adaptive cultivar was not affected by salt treatments (Barbieri et al., 2012). The authors suggested that a reduced stomatal density, high ascorbate concentrations and polyphenol oxidase activity coordinately contribute to enhance WUE under salt stress. Abiotic stress has been linked to plant hormonal pathways such as abscisic acid (ABA), a phytohormone designated as a key regulator in the activation of osmotic stress-responsive genes, but additionally, receptor-like kinases (RLKs) play essential roles in plant growth, development and responses to environmental stresses (Ouyang et al., 2010; Vu et al., 2020). For example, Ouyang et al. (2010) reported that the expression of a putative RLK encoding gene, *OsSIK1*, was mainly induced by salt in rice, and affected stomatal density in the abaxial and adaxial leaf epidermis.

In Figure 3, a positive correlation can be clearly observed between the number of stomata and transpiration. Hence, total transpiration rates are reduced by reduced leaf area and reduced stomatal density due to high Na^+ concentrations, thereby affecting biomass-WUE. In summary, our results suggest that sufficient K supply plays an important role for increasing biomass-WUE under saline environments by increasing biomass production, which is also supported by conducted preliminary experiments (Figure 2S), while salt stress decreases transpiration. However, more research on the physiological mechanisms with underlying molecular responses and hormonal signalling is needed to fully understand this interesting phenomenon in quinoa.

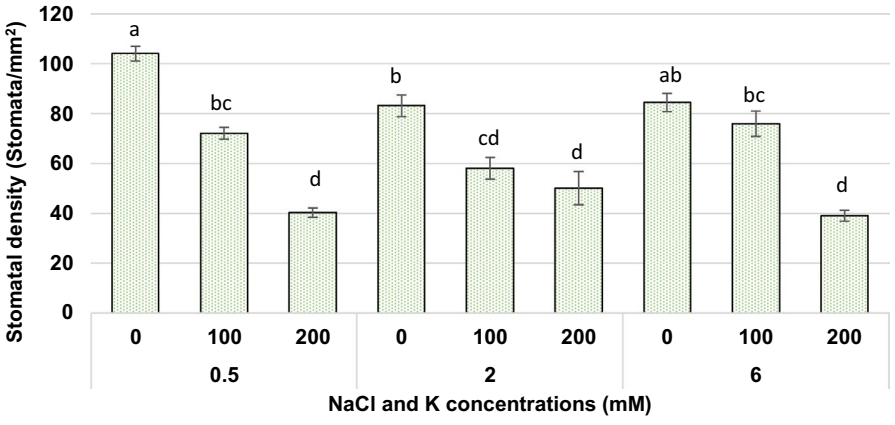

FIGURE 2 Stomatal density in quinoa cultivated under different NaCl (first row in the bottom of figure) and K (second row in the bottom of figure) concentrations (mM). Columns represent mean ± *SE* values of three biological replicates per treatment. Significant differences ($p \leq .05$) between treatments are indicated by different letters

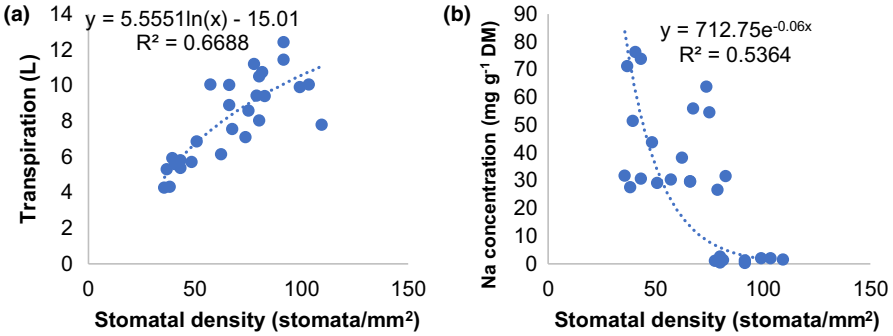

FIGURE 3 Correlation analysis between plant transpiration and stomatal density (a), and between sodium concentration in shoots and stomatal density (b)

TABLE 3 Leaf gas exchange, enzyme activity and hydrogen peroxide concentrations in quinoa cultivated under different NaCl and K concentrations

K (mM)	NaCl (mM)	A (μmol m^{-2} s^{-1})	E_L (mmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	CAT (μmol H_2O_2 g^{-1} min^{-1})	APX (μmol H_2O_2 g^{-1} min^{-1})	H_2O_2 (μmol/g)
0.5	0	14.05 ± 2.18a	4.02 ± 1.24a	526.59 ± 194.81a	1,922.52 ± 200.46a	1.99 ± 0.64b	3.06 ± 0.65a
2		17.78 ± 2.77a	3.47 ± 0.92a	418.61 ± 165.56a	1,175.18 ± 181.21a	5.11 ± 0.69a	2.96 ± 0.56a
6		15.64 ± 0.88a	2.45 ± 0.31a	249.8 ± 39.46a	1,550.88 ± 248.75a	4.49 ± 0.09ab	2.22 ± 0.17a
0.5	100	16.17 ± 1.34a	2.82 ± 0.55a	306.57 ± 76.38a	1,452.17 ± 300.24a	5.06 ± 0.59a	2.49 ± 0.31a
2		13.87 ± 1.87a	2.08 ± 0.5a	204.95 ± 59.39a	1,140.66 ± 200.32a	4.22 ± 0.13ab	2.04 ± 0.05a
6		12.82 ± 2.32a	2.06 ± 0.52a	209.34 ± 63.63a	1,072.35 ± 18.5a	4.06 ± 0.33ab	2.05 ± 0.25a
0.5	200	13.72 ± 0.9a	2.22 ± 0.22a	222.74 ± 23.98a	1,596.04 ± 199.76a	4.42 ± 0.78ab	1.75 ± 0.22a
2		13.76 ± 0.77a	1.98 ± 0.22a	190.52 ± 23.95a	1,415.2 ± 74.28a	4.5 ± 0.12ab	2.24 ± 0.91a
6		11.73 ± 0.82a	1.77 ± 0.18a	165.82 ± 20.96a	1,768.22 ± 181.99a	4.05 ± 0.88ab	2.88 ± 0.3a

Note: Values represent mean ± *SE* of three biological replicates per treatment. Means followed by the same letter are not significantly different from each other ($p \geq .05$).

Abbreviations: A, assimilation rate; APX, ascorbate peroxidase activity; CAT, catalase activity; EL, leaf transpiration rate; gs, stomatal conductance; H_2O_2 = hydrogen peroxide.

3.4 | Leaf gas exchange

Transpirational fluxes are influenced by stomatal density, which was shown to be affected by K supply and salt stress and stomatal aperture. Hence, transpiration rates were measured by leaf gas exchange. In contrast to stomatal density, leaf transpiration rates (E_L) were not affected by K supply ($p = .2136$). Analysing the factor K, at 6 mM K the lowest value of 2.10 mmol m^{-2} s^{-1} was measured, while at 0.5 mM K the highest E_L of 3.02 mmol m^{-2} s^{-1} was achieved. In the same way, stomatal conductance (g_s) was not significantly affected by K with the greatest value 351.97 mmol m^{-2} s^{-1} at 0.5 mM K and the lowest 208.32 mmol m^{-2} s^{-1} at 6 mM K. This contradicts some studies where the stomatal conductance is reported to decrease when K supply is deficient (Jákli et al., 2017; Terry & Ulrich, 1973; Zhao et al., 2001). However, there is also a consensus that under well-watered conditions which are in our study equivalent to non-saline conditions, stomatal functioning is well maintained even when leaf K^+ concentrations are below the critical values for photosynthesis (Tränkner et al., 2018). The role of K^+ in guard cell regulation is well documented (Fischer, 1968; Humble & Raschke, 1971). Being the major inorganic osmolyte, K^+ drives the changes in guard cell turgor that are required for stomatal movement. In our study, K^+ translocation from old leaves into young growing leaves might have increased K^+ concentrations in the measured leaf, thereby contributing to functional stomatal movement. Irrespective of K concentrations, salt stress induced a decrease in E_L ($p = .0442$). Highest E_L was obtained in the plants cultivated under non-saline conditions with 3.31 mmol m^{-2} s^{-1}, decreasing to 2.32 mmol m^{-2} s^{-1} at 100 mM NaCl and then to 1.99 mmol m^{-2} s^{-1} at 200 mM NaCl. About the interaction of both factors, K and NaCl, there was no significance difference ($p = .8749$). In the same direction, Razzaghi et al. (2011) also reported a decrease in E_L and g_s due to increased salinity. The reduction in E_L and g_s due to salinity can be associated with an increase in ABA production inducing stomatal closure and/or increased levels of H_2O_2. Salt stress causes hyperosmotic stress resulting in a production of ABA. However, ABA has been shown to respond to salinity mainly in glycophytes, where its concentration was two- to threefold increased due to salinity (Hedrich & Shabala, 2018). In halophytes, including quinoa, the ABA concentrations remained almost constant (Cocozza et al., 2013; Lavini et al., 2014), which might indicate that regulation of stomatal aperture operates over a very low ABA concentration range. Regarding the assimilation rate (A), the K factor did not have a defined trend, while the NaCl factor, although without significant differences, decreased as salinity increased, with the highest value of 17.78 μmol m^{-2} s^{-1} with 2 mM K and in the absence of salt, while the lowest value (11.73 μmol m^{-2} s^{-1}) was obtained with

a concentration of 6 mM K and 200 mM NaCl (Table 3). In line with these findings, Koyro et al. (2013) reported that an increase in NaCl concentrations caused significant changes in various gas exchange parameters and low CO_2-assimilation rate which led to the development of ROS due to over-reduction of reaction centres.

3.5 | ROS scavenging enzyme activity

Stress factors, like salinity, can severely affect plant metabolism and create an imbalance in the production and scavenging of ROS such as H_2O_2; however, under stress conditions plants can respond producing enzymatic and non-enzymatic antioxidants such as catalase, ascorbate peroxidase among others to enhance the scavenging of ROS (Amjad et al., 2015). Regarding the H_2O_2 concentration, the factors showed no significance difference (K: $p = .9916$; NaCl: $p = .3140$; interaction: $p = .2756$). However, with increasing K concentration, the values of H_2O_2 in the plants decreased. This trend was also observed in other preliminary experiments where plants supplied with 2 mM K showed a H_2O_2 decrease when compared to lower K concentrations (Figure 2S). The H_2O_2 values at different NaCl concentrations were also similar, ranging from 2.19 µmol/g FM to 2.74 µmol/g FM at 100 mM NaCl and 0 mM NaCl, respectively (Table 3).

Nevertheless, according to the analysis of variance, CAT showed no significant difference for both factors, K ($p = .0578$) and NaCl concentration ($p = .0631$). Plants cultivated with 0.5 mM K under non-saline conditions showed the highest CAT with a mean value of 1922.52 µmol H_2O_2 g^{-1} min^{-1}. A high activity (1768.22 µmol H_2O_2 g^{-1} min^{-1}) was also measured in plants with 200 mM NaCl and 6 mM K. The lowest values were achieved in plants cultivated with K concentrations between 2 mM K and 6 mM K at 100 mM NaCl with activities 1,140.66 µmol H_2O_2 g^{-1} min^{-1} and 1,072.35 µmol H_2O_2 g^{-1} min^{-1}, respectively (Table 3). It can be noted that increased CAT occurs under non-saline conditions and at 200 mM NaCl, as well as with K deficiency (0.5 mM K); this suggests that under K^+ deficiency, moderate salt concentration can help against oxidative stress, since Na^+ can carry out some non-specific K^+ functions as mentioned above. In contrast to CAT activity, an increased APX activity was determined in plants cultivated at 100 mM NaCl. This increase in APX activity in quinoa under saline conditions was also observed by Amjad et al. (2015). The reason for this could be based on the equilibrium between CAT, glutathione peroxidase (GPX) and APX as important H_2O_2 scavenging enzymes (Sofo et al., 2015). Since reduced CAT activity was measured at 100 mM NaCl, the APX value had to be upregulated to maintain this equilibrium. A similar behaviour was observed regarding K concentrations, when comparing CAT and APX activities, where the CAT values were in exactly the opposite order to the APX results. For example, in the plants with 0.5 mM K in the absence of salt the highest CAT activity (1,922.52 µmol H_2O_2 g^{-1} min^{-1}) but the lowest APX activity (1.99 µmol H_2O_2 g^{-1} min^{-1}) was determined, while at 2 mM K in the absence of salt a relatively low CAT activity (1,175.18 µmol H_2O_2 g^{-1} min^{-1}) and the highest APX activity (5.11 µmol H_2O_2 g^{-1} min^{-1}) were measured.

According to these results, although there was no statistically significant difference between treatments in both factors, K deficiency led to a slight increase in the production of ROS, such as H_2O_2, which resulted in inhibition of plant growth. This is also supported by CAT measurements, since at 0.5 mM K the highest CAT was determined, as was the increased accumulation of H_2O_2 (Table 3). Under non-stress conditions, these ROS are eliminated by non-enzymatic and enzymatic antioxidants, whereas under salt stress this ability is reduced by the increased production of ROS and causes oxidative stress and finally to cell death (Sofo et al., 2015); however, halophytes have the ability to cope with saline stress, where moderate salinity does not represent a major change in plant physiology. In addition, it is possible to enhance the tolerance to salinity in salt-tolerant like quinoa through different methods such as seed priming (Moreno et al., 2018; Yang et al., 2018), improving the physiological response of plants to the negative effects of salt stress.

3.6 | Principal component analysis – PCA

As can be seen in Figure 4 from the PCA, the horizontal axis PC1 has the most variation with 46.2%, while the vertical axis PC2 explains

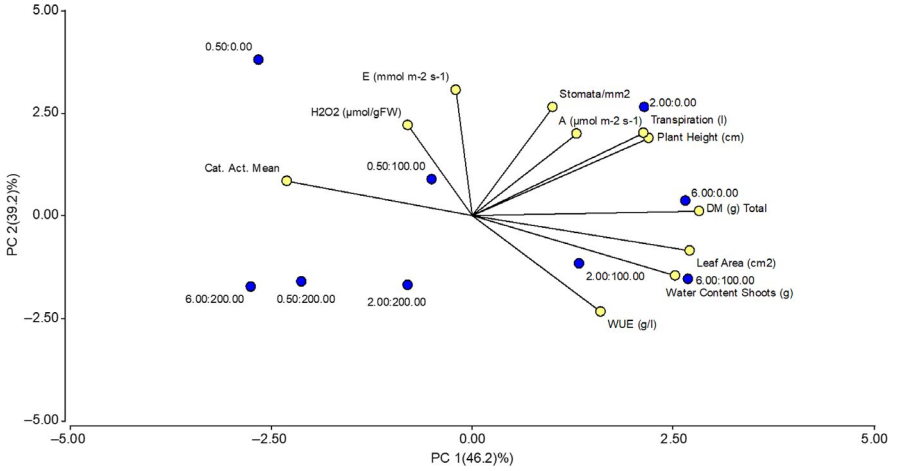

FIGURE 4 Principal component analysis (PCA). Yellow dots represent the different dependent variables and the blue dots the treatments where the first value is related to the K treatment and the second one is related to the NaCl treatment

39.2% of the variation. The variables CAT and H_2O_2 are in the opposite direction to the growth parameters and correlate both negatively with growth parameters. In this analysis, it can be seen that CAT activity and H_2O_2 concentration are more correlated with high salinity and/or K deficiency, meaning that under stress conditions, the production of H_2O_2 is increased and the activity of CAT enhanced. Growth parameters are directly correlated with sufficient K supply and low salinity. Additionally, the variables total transpiration, assimilation rate, stomatal density and plant height are close to each other, being positively correlated between them, and in the direction of treatment 2 mM K and non-saline conditions. Therefore, under this treatment the values of those variables are higher. Furthermore, for example assimilation rate and stomatal density are close to each other, being positively correlated (0.51), in the same way stomatal density with total transpiration (0.83; Table 1S). The DM variable is in the direction of 6 mM K treatment and non-saline conditions; therefore, the highest values are obtained under this treatment. The variables WUE, water content and leaf area are benefited by a concentration between 2 mM K and 6 mM K at 100 mM NaCl, with a correlation between WUE and leaf area of 0.71, while between leaf area and water content there is a correlation of 0.96. This is because, as discussed above, under saline conditions, water content increases due to the Na^+ proprieties, but this also has a direct effect on the leaf area which may be due to cell expansion due to increased succulence.

4 | CONCLUSIONS

Salinity and different K concentrations had a significant effect on growth and physiological parameters in quinoa. At K concentrations between 2 and 6 mM, the biomass production and WUE were increased. WUE also increased under saline conditions due to a decrease in plant transpiration. Transpiration and stomatal density were positively correlated, so an increase in salinity dramatically decreased transpiration as a consequence of a significant decrease in stomatal density. Quinoa is able to take up more K^+ under saline conditions to maintain the K^+/Na^+ ratio as a mechanism to avoid salt stress; therefore, K^+ is important to reduce possible damage caused by salinity and at the same time increase crop productivity. On the other hand, under K^+ deficiency conditions, Na^+ is also capable of carrying certain non-specific K^+ functions, such as osmotic regulation, and thus ensuring the survival of plants. Our promising results are the basis for further studies, considering mineral nutrition as an important key for salt tolerance and thus optimizing crop productivity under saline conditions.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Anna-Maria Trappe for her very important support in taking care of the plants and carrying out measurements in the laboratory. We also thank Mrs. Kirsten Fladung and Mrs. Ulrike Kierbaum (Institute of Applied Plant Nutrition, University of Göttingen) and Birgit Lippmann (Institute of Botany, Leibniz University Hanover) for their valuable technical support. Funding of K + S Minerals and Agriculture is acknowledged. Open Access funding enabled and organized by ProjektDEAL.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

A.E.T. was involved in conceptualization, methodology, software, validation, formal analysis, data curation, project administration, investigation, supervision and writing—original draft preparation; M.T. was involved in resources, funding acquisition, writing—review and editing, visualization and supervision; J.P. was involved in resources, writing—review and editing, and visualization. All authors have read and agreed to the published version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Turcios AE, Papenbrock J, Tränkner M. Potassium, an important element to improve water use efficiency and growth parameters in quinoa (*Chenopodium quinoa*) under saline conditions. *J Agro Crop Sci*. 2021;207:618–630. https://doi.org/10.1111/jac.12477