Morphophysiological responses of two *Chenopodium quinoa* genotypes to salinity in a hydroponic system

Abstract. The *Chenopodium quinoa* is poised to be a promising species for future food security and combating climate change because of its nutritional content and its halophytic peculiarity. This study focuses on the differential responses of salt-tolerant (*Chadmo*) and salt-sensitive (*Kankolla*) under control (CK) and 400 mM NaCl in five temporal dimensions (1/2, 1, 3, 6, and 24 h post-treatment time points). Agronomic features assessed include root/shoot dry biomass ratio, relative water content (RWC), membrane stability index (MSI), total chlorophyll (CHL), and adaxial epidermal bladder cells (EBC). The results indicated a reduction in dry biomass for both genotypes after treatment with *Chadmo* and *Kankolla* at 46.96% and 73.07%, respectively. Similarly, a significant reduction in the RWC with *Chadmo* at 16.69 % and *Kankolla* at 13.19% was detected. Under 400 mM NaCl condition and CK, *Chadmo's* average net photosynthetic rate reduced from 15.73±2.97 to 13.02±2.75 µmol CO_2 $m^{-1}s^{-1}$, indicating 17.24% reduction while in the *Kankolla,* the reduction from was 13.43±4.12 to 8.34±3.16 µmol CO_2 $m^{-1}s^{-1}$ representing 37.92% decline. In addition, this study showed a significant difference ($p<0.05$) being identified on ANOVA and Tukey analyses in root/shoot dry biomass ratio, relative water content (RWC), membrane stability index (MSI), total chlorophyll (CHL), and adaxial epidermal bladder cells (EBC). Those measurement criteria increased by 14.45% and 3.45% in *Chadmo* and *Kankolla*, respectively, from the CK to 400 mM NaCl. Using these morpho-physiological responses to salinity, *Chadmo* proved to be the better-performing genotype when exposed to 400 mM NaCl and hence identified as the salt-tolerant genotype.

Keywords: Climate change, halophytes, nutrients, quinoa, resilience, salinity, salt-tolerant

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1. INTRODUCTION

The global population is marching towards an unprecedented proportion amidst the challenges of climate change, seemingly highly engineered by anthropogenic influences. Concomitantly, demands for food will have to be increased by 70% to provide for the ~ 9.8 and 11.2 billion people by the years 2050 and 2100, respectively (15; 70; 78). Globally, salinity affects about 6% and 20% of the total and irrigated lands, respectively (15; 73). Climate change models and other anthropogenic factors poignantly navigate at increasing aggregate global soil salinization by 50% in 2050 (15; 83). While some plants have adapted to a different mechanism to escape the impact of excess salt exposure, others remained susceptible and unable to continue their life cycle, as in the case of the glycophytes. Plants that survived high salinity devised the mechanisms of avoidance, exclusion, and compartmentalization of the ions that increase the toxicity of cells (8; 70). Halophytic plants can grow and produce viable seeds at ≥200 mM NaCl concentrations, lethal to about 99% of other plant species (19; 64). High salt concentrations in the soil or other growth media will cause hyperosmotic stress in roots and other structures (12; 24). It efficiently decreases the plant's ability to absorb water because of osmotic stress. Once absorbed, the water's ionic constituents Na+ and Cl- adversely affect the metabolic activities and reduce photosynthetic efficiency (21; 40).

Quinoa has salt bladders that have approximately 1000-fold more volume space than regular epidermal cells. Hence, it can potentially load and sequester more ions and osmolytes than adjacent cells and plants without salt glands (6; 8; 69). Moreover, its ability to survive at salinity levels even

higher than that of seawater makes it incomparable and more suitable than some other halophytes under such abiotic stress (28; 48; 55). Quinoa can exclude salts and physiologically adjust them to minimize their effects in high concentrations (6; 19; 55). Jacobsen et al (34) observed that the quinoa yield was the highest at 100 to 200 mM NaCl and, after that, decreased. Further support to this was provided by Hariadi et al (28), who recorded significant inhibitory effects on seed germination at concentrations higher than 400 mM NaCl, while optimal plant growth was obtained between 100 and 200 mM NaCl with *Titicaca* over a 70-day growth period. Gómez-Pando et al (26) screened 182 quinoa accessions for salt tolerance and found that 25% exhibited greater than a 60% germination rate at 250 mM NaCl for seven days. In a pot experiment, these 15 accessions were further tested at 300 and 340 mM NaCl. The results indicated that 13 accessions showed a reduction in growth, while two grew 1.79 to 11% higher than the CK. Morales et al (44) also observed that at 300 mM NaCl, quinoa cultivars *Chipaya* and *Ollague* showed a decrease in fresh weight, but at 450 mM NaCl, they sustained 50 and 40% higher transpiration rates than the CK, respectively.

Quinoa is becoming an important food crop because of its high nutritional composition and potential to grow in environmental stresses that would be detrimental to many other plants (18; 81). For these attributes, 2013 was recognized as the '*International Year of the Quinoa'* by the Food and Agriculture Organisation (FAO) of the United Nations (UN) (18; 56). This study determines the morphological and physiological responses of two contrasting quinoa genotypes (salt-tolerant *Chadmo* and salt-sensitive *Kankolla*) used for salinity under the hydroponic system.

2. MATERIALS AND METHODS

2.1 Plant material, Seedling growth, and treatment

Two contrasting genotypes (salt-tolerant {Chilean genotype} and salt-sensitive {Peruvian genotype}) were used in this study. The United States Department of Agriculture (USDA) in Washington, USA, kindly provided the seeds (80). Fresh seeds were germinated in PINDSTRUP substrate (dark sphagnum peat mixed with 30% natural, fibrous material, 50g of micronutrients). At two true leaf stages (~10 days), the seedlings were transferred into the hydroponic system containing water. After one day, the Hoagland solution was added. Then five days later, salt was added incrementally (50 mM NaCl day^{-1}) to avoid osmotic shock and damage to the root until the 400 mM NaCl concentration threshold of treatment was achieved (4; 28; 32). As necessary, a consistent level of solution (7L) was maintained by adding nutrient solution with the respective NaCl concentrations. The plants were arranged in a complete randomized block design in the hydroponic box, with six biological replicates per treatment, and grown in a greenhouse at 24±2 ^{o}C with 65-70% relative humidity and at a 16 h light (400 u mol m^{-2} s^{-1})/8 h dark cycle. Therefore, except for the physiological parameters, stomatal index, and epidermal bladder cell measurements, which were done on the 45th day. All other samples were harvested immediately at the end of the treatment (400 mM NaCl, 24th day) at the different time points (0 (CK- untreated plants that were grown parallel to the treated plants),1/2, 1, 3, 6 and 24 h post-treatment time points).

2.2 Morpho-physiological analysis

2.2 (a)Morphological analysis

2.2 (a) (i) Dry biomass. Dry weight (DW) was determined by apportioning three plants each from CK and 400 mM NaCl into the shoot and root. Samples were weighed and then wrapped with aluminum foil and oven-dried at 105°C for 19 min, followed by 80°C for 24 h. After constant mass was achieved, samples were removed, and the dry weight of the shoot and root were measured. Leaf samples were digested to quantify Mg^{2+}, Ca^{2+}, Na^{+}, and K^{+} (37; 42; 61).

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2.2 (a) (ii) Relative Water Content (RWC). Leaves were harvested from midday for relative water content, and the fresh weight (FW) was measured. Leaves were floated in ddH_2O for 4 h and then reweighed to determine the turgid weight (TW) after being sapped with a paper towel to remove the surface water. Next, dry weight (DW) was determined: Samples were weighed and then wrapped with aluminum foil and oven-dried at 105°C for 19 min, followed by 80°C for 24 h. After constant mass was achieved, samples were removed, and the dry weight of the samples was measured. The RWC was determined with the following formula: RWC=(TFW-DW/TW-DW)x100, where TFW is total fresh weight, DW is dried weight, and TW is turgid weight in g (27; 37; 41; 59).

2.2 (a) (iii) Membrane Stability Index (MSI). Leaves were harvested from each plant during the midday period and washed with tap H_2O once. Then the leaves were washed twice with dH_2O and finally once with ddH_2O to remove surface salts and contaminants. Leaves were then sapped with a paper towel to remove excess water, then discs (8mm) were cut with a cork borer, weighed (~0.1g) in each set, and placed into two separate 15 mL falcon tubes to which 10 mL of ddH_2O was added. One set of tubes was placed in a water bath at 40°C for 30 minutes. At the same time, the other was placed in another at 100°C for 15 minutes. After heating, both sets of tubes were allowed to cool, and then the leaf leachates' electrical conductivity (EC) was determined using an Extech Instruments, ExStik11 EC/Sal/TDS meter. The membrane stability index (MSI) was then determined by applying the formula: MSI = 1- (C_1/C_2) x 100, where C_1 and C_2 = Electrical conductivity of leaf leachate at 40 and 100 ^{o}C, respectively (27; 41; 54; 59).

2.2 (a) (iv) The chlorophyll and carotenoid contents. Chlorophyll and carotenoid were estimated via the non-maceration method. Leaf samples (20 mg) (were immersed in 10 mL of dimethyl sulphoxide (DMSO) at 65 ^{o}C for 4 h and then cooled (31; 76). Absorbance was read spectrophotometrically at 645, 663, and 470 nm with DMSO as the blank, chlorophyll (3), and carotenoid (38) were quantified.

2.2 (a) (v) The Epidermal Bladder Cells (EBC) and Stomatal Index. Leaves (six leaves per plant: three young - third to fifth from the tip and three mature - the lowest three non-senescing leaves) were taken from three plants per treatment and CK of both genotypes. The leaves were sampled before flowering (□45 days), allowing for more plant maturity to assess the differential distribution with time. For EBCs, they were observed and photographed under an optical microscope (x100), after which the epidermal bladder cell distribution was evaluated (26; 48). For the stomatal index, a thin layer of transparent nail polish was evenly applied to both the adaxial and abaxial epidermal surfaces. After drying (~20 min), the nail polish blotting film, with the respective epidermal imprints (~ 1cm^2), was carefully removed and placed on microscopic glass slides and then covered with transparent adhesive tape. The imprints were then carefully observed 100 times under a microscope, and the total epidermal and stomatal cells were determined through digital photography (23; 39; 48; 85). The stomatal index was determined using the developed formula: SI (%) = N_s (E_c + N_s) x 100, where N_S is the total number of stomata in view, and E_C is the total number of epidermal cells (63).

2.3 measurements

The LICOR 6800 Portable Photosynthesis System (LiCor instrument, Inc., NE, USA) was used to measure the net photosynthesis, transpiration rate, intercellular carbon dioxide, and stomatal conductance. That measurement in three randomly selected fully expanded leaves at three measurements per leaf for each treatment for each plant (~45 days). Measurements were taken at ambient CO_2 concentration (380 μmol mol^{-1}), light exposure (400 μmol m^{-2}s^{-1}), relative humidity controlled at □65%, and leaf chamber temperature stable at 24 ^{o}C. The lights in the glasshouse were

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kept on for about 4 h before and during the measurement period (10:00-14:00 h) to maintain a relatively stable light level to give consistency in measurements (49; 65).

3. DATA ANALYSIS

The experiment was conducted in a completely randomized block design with three biological replicates per treatment. The data were subjected to analysis of variance test (ANOVA) and Tukey posthoc analyses expressed as the mean of the three replicates (mean±SD), and significance among treatments and varieties for morphological and physiological significance was checked at $p<0.05$ and $p<0.01$. The Statistical Package for Social Sciences (Version 21 for Windows, SPSS Inc., New York, NY, USA) and Minitab Statistical Software (Version 19 for Windows, Pennsylvania, USA) were used to perform the analysis.

4. RESULTS

4.1 Biomass – root/shoot ratio

The shoot/root dry weight ratio was similar for the *Chadmo* and *Kankolla* genotypes at the CK, having 8.43 and 8.54, respectively. However, after treatment, both indicated a significant reduction to 4.47 and 2.3, representing 46.96% and 73.07% in *Chadmo* and *Kankolla*, respectively (Figure 1). The analysis of variance identified no significant difference ($p<0.05$) between the CKs of the two genotypes. However, a significant difference ($p<0.05$) was noted between the CK and 400 mM NaCl treatment for both genotypes. Moreover, a significant difference ($p<0.05$) was also observed between the treated plants for both genotypes.

Figure 1. Response of *Chadmo* and *Kankolla* to CK and 400 mM NaCl in root/shoot. Mean ± SD (n=3). Different letters indicate a significant difference at $p<0.05$ in *Chadmo* and *Kankolla*, respectively.

4.2 Relative Water Content (RWC)

Chadmo had a higher RWC in control with 98.21%, and the treated plants with 81.56%. For the *Kankolla*, the RWC for control was 82.25%, and for the treated, it was 71.40%. Comparatively, there was a higher reduction in the plants' RWC from the control to treated conditions in the *Chadmo* and *Kankolla* genotypes, with 16.69 and 13.19%, respectively. Significant differences were observed in *Chadmo* and *Kankolla* between the CK and 400 mM NaCl, respectively (Figure 2).

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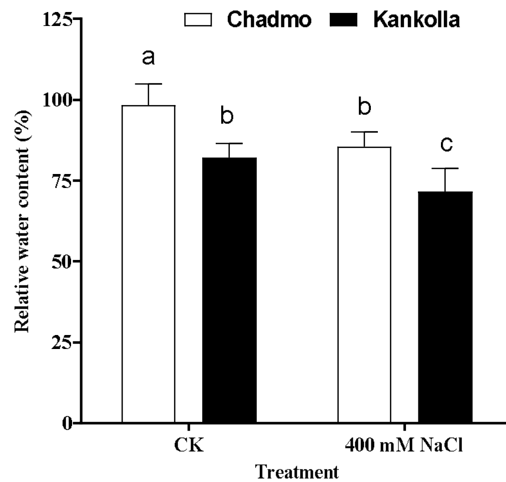

Figure 2. Response of *Chadmo* and *Kankolla* to 400 mM NaCl and control: Relative Water Content (RWC). Mean ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*, respectively.

4.3 Membrane Stability Index (MSI)

In both genotypes, MSI decreased with salinity stress at treated conditions. Notably, the MSI for the *Chadmo* was higher under control and treated at 85.49 and 55.16%, respectively. As a comparison, the Kankolla at the control and treatment was 80.40 and 40.99%, respectively. For MSI between control and treatment for the *Chadmo* and *Kankolla*, there was a 35.48 and 49.01% reduction, respectively (Figure 3). MSI between genotypes was not significant (p<0.01) at the level of the control while under treatment, the *Kankolla* being ~ 10% lower than the *Chadmo*. On the contrary, a significant difference was observed (p<0.01) between the two genotypes under treatment, with the *Chadmo* having about 15% higher than the *Kankolla*.

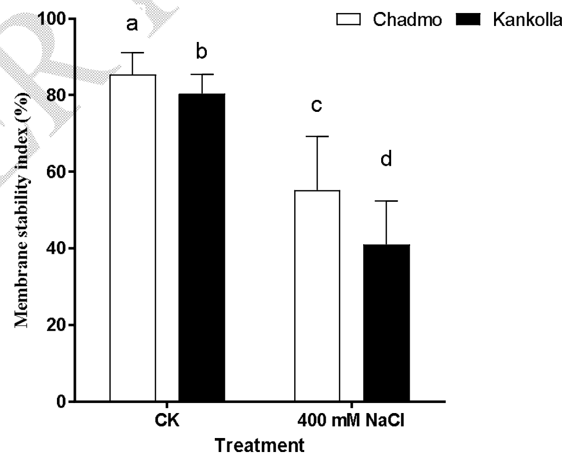

Figure 3. Response of *Kankolla* and salt-tolerant in membrane stability index to CK and 400 mM NaCl. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*, respectively.

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4.4 Photosynthetic pigments

Total chlorophyll and carotenoid contents displayed a similar pattern in both genotypes for the CK and 400 mM NaCl; a decreasing trend with increasing salt concentration was observed. The chlorophyll content was 35.64±1.61 and 29.96 ±4.79 mgg^{-1} and 35.35±1.00 and 21.62±2.16 mgg^{-1} for *Chadmo* and *Kankolla* at the CK and 400 mM NaCl (Figure 4 (a)). Total chlorophyll decreased slightly by 12. 86% between the CK and 400 mM NaCl for the *Chadmo*, representing no significant difference ($p<0.05$). However, a 30% decrease was observed between the CK and 400 mM NaCl for the *Kankolla*, indicating a significant difference ($p<0.05$). Besides, a significant difference ($p<0.05$) was observed for both *Chadmo* and *Kankolla* genotypes under 400 mM NaCl. For carotenoid, *Chadmo* had 2.69±0.21 and 2.03±0.38 mgg^{-1}, *Kankolla* had 2.61±0.33 and 1.15±0.11 mgg^{-1} at the CK and 400 mM NaCl, respectively (Figure 4 (b)). Moreover, the decrease in carotenoid for the *Chadmo* between CK and 400 mM NaCl was 49.24%, while for the *Kankolla*; it was 57.97%.

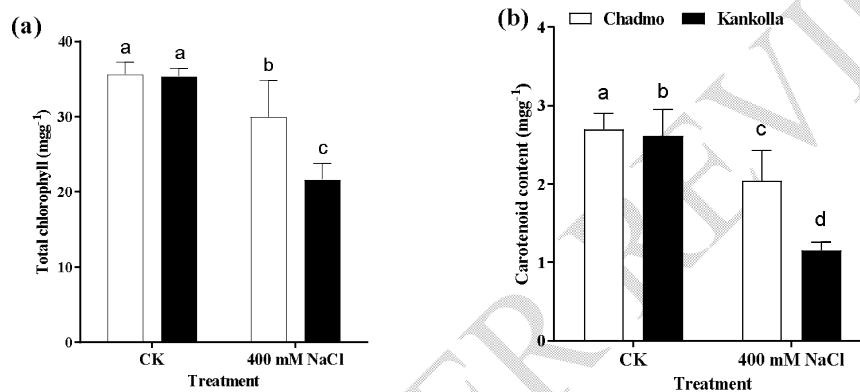

Figure 4. Response of *Chadmo* and *Kankolla* to 400 mM NaCl and control in contents of (a) total chlorophyll and (b) carotenoid. Means ± SD (n=4). Different letters indicate a significant difference at $p<0.05$ in *Chadmo* and *Kankolla*, respectively.

4.5 Epidermal Bladder Cells (EBC)

Chadmo displayed a numerical increase in the epidermal bladder cells in both the adaxial and abaxial surfaces at 400 mM NaCl compared with the CK (Figure 5 (a)). However, no significant difference ($p<0.05$) was found between the adaxial and abaxial surfaces. EBC average increase between the CK and 400 mM NaCl *Chadmo* was from 62.30±18.9 to 68.90 ±16.40 cells mm^{-2} representing a 10.59% increase. Adaxially, the maximum and minimum EBCs in the treated *Chadmo* were 81 and 5 cells $mm^{-2,}$ while for the CK, they were 77 and 9 cells mm^{-2}, respectively. On the abaxial surface, the maximum and minimum EBCs observed for the treated and CK *Chadmo* were 66, 81, 5, and 8 cells mm^{-2}, respectively. On the adaxial surface on *Chadmo*, EBCs increased from 31.75±15.89 to 33.10±22.73 cells mm^{-2}, while for the abaxial surface, the increase was from 31.25±18.9 to 35.80 ±16.40 cells mm^{-2}, representing 14.46 and 4.25% increase, respectively.

As with *Chadmo*, *Kankolla* also indicated increased EBCs on both the adaxial and abaxial surfaces, with no significant ($p<0.05$) difference in CK and treated. The adaxial surface increase was found to be 36.51±11.34 to 37.76±13.44 cells mm^{-2}. In comparison, on the abaxial surface, the increase was from 36.20±11.97 to 39.13±12.63 cells mm^{-2}. The increase in EBCs in the Kankolla was 3.45 and 8.10% in the adaxial and abaxial surfaces, respectively. The maximum and minimum EBCs in the adaxial surface in the CK and treated were 58, 62, 17, and 12 cells mm^{-2}; for the abaxial surface, they were 66, 61, 13, and 19 cells mm^{-2}, respectively (Figure 5(b)).

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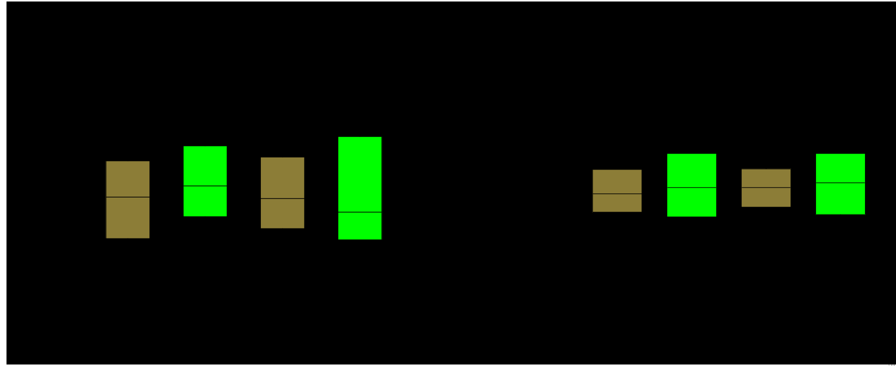

Figure 5. Epidermal bladder cells (ECBs) distribution in (a) *Chadmo* and (b) *Kankolla* at CK and treated (400 mM NaCl) in the adaxial and abaxial surfaces. Mean ± SD (n=60). Different letters indicate a significant difference at $p<0.05$ in *Chadmo* and *Kankolla*, respectively.

4.6 Stomatal index

The stomatal density and index displayed a decreasing pattern in both *Chadmo* and *Kankolla* from CK to 400 mM NaCl treatment. In *Chadmo*, stomatal density decreased from 199.98±26.88 mm^{-2} to 179.33±17.69 mm^{-2} on adaxial surface and 243.74±27.26 mm^{-2} to 224.04±26.80 mm^{-2} on abaxial surface from CK to the treated *Chadmo*. This decrease represented 10.33 and 8.08% between the CK and treated *Chadmo* in adaxial and abaxial surfaces (Figure 6 (a)). A similar trend was observed in the stomatal indices of both the adaxial and abaxial surfaces of *Chadmo*. In the stomatal index, the decrease was from 16.06 to 13.61% and 15.91 to 14.68% in adaxial and abaxial surfaces in CK and treated *Chadmo*, respectively. All the decreases from the CK to treated have been noted to have a significant difference at $p<0.05$ in ANOVA and Tukey analyses (Figure 6 (b)).

Kankolla's alterations in stomatal density in response to the treatment were more pronounced than *Chadmo's*. The decrease from the adaxial and abaxial was 211.29±34.90 to 118.25±39.65 mm^{-2} and 270±54.09 to 84.05±27.75 mm^{-2} from the CK to treated plants representing 40.03 and 68.98% decrease (Figure 6 (c)). Similarly, the stomatal index declined from 12.38 to 10.96% and 14.92 to 8.33% from the CK to 400 mM NaCl in the adaxial and abaxial surfaces, respectively. This decrease in the stomatal index was 11.46 and 44.28% between the adaxial and abaxial surfaces between the CK and treated *Kankolla,* respectively (Figure 6 (d)). In addition, ANOVA and post hoc Tukey analyses identified significant differences in the stomatal density and stomatal index in the CK and the treated plants at the adaxial and abaxial surfaces (Figure 6(d)).

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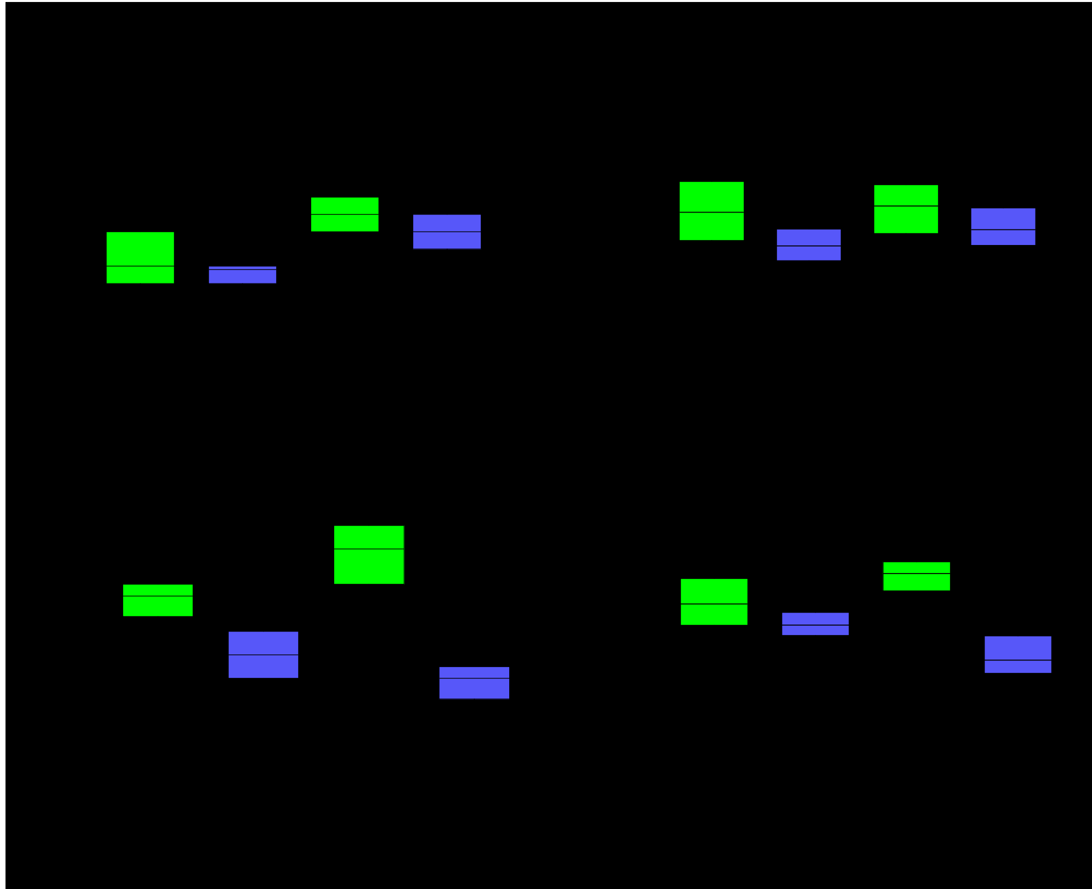

Figure 6. (a) Stomatal density on *Chadmo*, (b) stomatal index on *Chadmo*, (c) stomatal density on *Kankolla*, and (d) stomatal index on *Kankolla* on CK and treated (400 mM NaCl) in the adaxial and abaxial surfaces. Means ± SD (n=60). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*, respectively.

4.7 Physiological response

The net photosynthesis (Pn), transpiration rate, stomatal conductance, and intercellular carbon dioxide measurements exhibited metabolism-reduction imposed by salt stress in both genotypes. While both genotypes displayed phenotypes of halophytic plants with reduced metabolic functions and attenuation in plant height, the *Kankolla* genotypes exhibited a higher percentage of reduction, displayed in chlorotic leaf spots and slight leaf curling (Figure 7 (a)). Under 400 mM NaCl condition and CK, *Chadmo's* average net photosynthetic rate reduced from 15.73±2.97 to 13.02±2.75 µmol CO_2 $m^{-1}s^{-1}$, indicating 17.24% reduction while in the *Kankolla,* the reduction from was 13.43±4.12 to 8.34±3.16 µmol CO_2 $m^{-1}s^{-1}$ representing 37.92% decline (Figure 8 (a)). Similarly, the transpiration rate reducing from CK and 400 mM NaCl treatment in *Chadmo* and *Kankolla* genotypes from 0.0068±0.001 to 0.0032±0.001 µmol of CO_2 m^{-2} s^{-1} and 0.0056±0.002 and 0.00323±0.001 µmol of CO_2 m^{-2} s^{-1}, respectively (Figure 8 (b)). Concerning stomatal conductance, the *Chadmo* had a higher exchange (1.16±0.55 µmol H_2O $m^{-1}s^{-1}$) than *Kankolla* (1.06±0.88 µmol H_2O $m^{-1}s^{-1}$). Moreover, this was reduced in the *Chadmo* and *Kankolla* to 0.41±0.22 and 0.14±0.17 µmol H_2O $m^{-1}s^{-1}$, representing 64.73 and 86.18% decline, respectively (Figure 8 (c)). Intercellular carbon dioxide measurement indicated a decrease from the CK to 400 mM NaCl, with the *Chadmo* and *Kankolla* genotypes exhibiting a reduction from 343.54±19.92 to 305.71±28.82 CO_2 µmol mol^{-1} and 341.08±26.43 and 279.59±60.37 CO_2 µmol mol^{-1} representing 11.01 and 18.02%, respectively (Figure 8 (d)). In addition, significant differences were identified by ANOVA analysis (p <0.05) in the net

photosynthesis, transpiration rate, stomatal conductance, and intercellular carbon dioxide between the control and treatment.

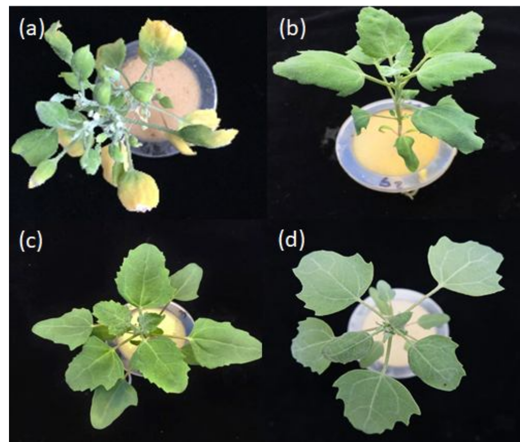

Figure 7. Phenotypic response to salinity in *Kankolla* (a) treated – curling and chlorotic leaves (b) control and salt-tolerant - *Chadmo* (c) treated (d) control.

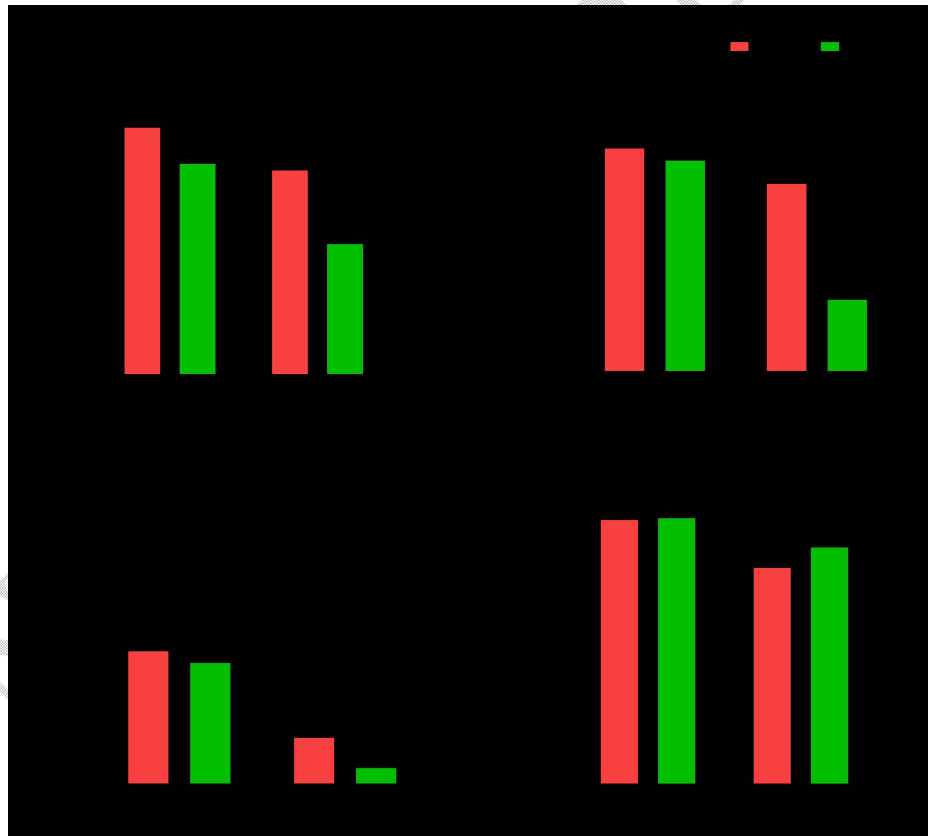

Figure 8. Physiological responses of *Chadmo* and *Kankolla* to CK and 400 mM NaCl (a) net photosynthesis (b) transpiration rate (c) stomatal conductance, and (c) intercellular CO_2. Means ± SD (n=6). Different letters indicate a significant difference at $p<0.05$ in *Chadmo* and *Kankolla*, respectively.

5. DISCUSSION

Reduction in dry biomass is common among plants under saline conditions and displayed further reduction as salinity increases up to the point of survival (1; 19; 50). This study posited that even though the *Chadmo* is deemed a more salt-tolerant genotype, salinity does impact the plant. In contrast, the plant survived; it is not completely void of the effects on its general physiology, which can eventually propel a reduction in dry biomass. Both genotypes showed a significant ($p<0.05$) decrease in the root/shoot dry biomass ratio between the CK and 400 mM NaCl, which indicates the effect salinity has on plant growth and development (20; 25; 36; 82). Shabala et al (69) identified a 50% reduction in biomass in the quinoa *cv 3706* grown in 400 mM NaCl compared with the control. Besides, Ruiz et al (58) observed a 60-70% root-to-shoot fresh weight ratio in the landraces *Villarrica* and *49* grown in 300 mM NaCl compared with the CK, while Gómez-Pando et al (26) identified a decrease in leaf and root dry mass per plant by 81.13 and 80% in accessions 154 and 19 respectively in 300 mM NaCl relative to the control. Interestingly, no significant difference ($p<0.05$) was observed in total plant dry weight across the treatments in *BO78* grown in 150 to 750 mM NaCl as compared with the control (48). Hariadi et al (28) contended that both shoot and root fresh weight decreased significantly by 20 and 50%, respectively, relative to the control in quinoa *cv 5206*.

Measuring relative water content in plants subjected to salinity stress is physiologically relevant to determine their tolerance level. Previous studies have outlined that under saline conditions, it adjusts their osmotic potential to maintain turgor pressure, increasing water content, particularly in the leaf (7; 9; 45). On the contrary, in this study, both varieties showed that RWC decreased significantly ($p<0.05$), with salt-tolerant *Chadmo* reducing by 17.44% and salt-sensitive *Kankolla* by 10.85% from the control to 400 mM NaCl. Plants curtail water loss by developing cutinized epidermal layer and facilitating stomatal closure in conjunction with accumulated ABA in the chloroplast and guard cells (47; 67; 84). Plant injury is another resultant effect of a highly salinized environment, and it can be in many forms, such as injury to roots, stem, and leaf cells. Therefore, plants responded by developing more robust membrane systems to avoid such damaging effects. Hence, one of the features to assess salinity tolerance is the level of injury experienced by the membrane through membrane stability index or electrolyte leakage, which measures the cell membrane injury caused by environmental stress (60; 71; 72). Comparatively, plants with reduced membrane stability index have a higher tolerance level for abiotic stress such as salinity and have been employed to screen for salinity tolerance in plants (5). In this study, we observed a significant difference ($p<0.01$) between salt-tolerant *Chadmo* and salt-sensitive *Kankolla,* with the latter having 13.53% MSI more than salt-tolerant *Chadmo*. No significant difference was observed between the controls of the two varieties at $p<0.001$. Previous studies have also related a reduction in MSI to plant salinity tolerance (16; 17; 60; 61; 68; 72).

Total chlorophyll and carotenoid contents decreased more at 400 NaCl than in the CK. Moreover, the reduction between the control and 400 mM NaCl in salt-sensitive *Chadmo* was insignificant, while for salt-sensitive *Kankolla*, a significant difference was identified by ANOVA at $p<0.05$. Leaf chlorosis, white spots/appearance, and leaf rolling were also observed in treated salt-sensitive *Kankolla*, providing further evidence that salinity impacts the biosynthesis of chlorophyll or is destructive post-synthesis (2; 11; 25; 33; 48; 53). Ruffino et al (57) reported a decrease in total chlorophyll in salt-treated as compared with the CK in quinoa cultivar *Sajama* seedlings over 21 days. They concluded that total chlorophyll decreased by 40.2, 25.5, and 38.9% after 6, 12, and 21 days of 250 mM NaCl compared with the CK. They posited that Chlorophyll b had a significant difference while total chlorophyll had no significant differences in reduction between the CK and 250 mM NaCl. Similarly, carotenoid contents in *Sajama* decreased between the CK and 250 mM NaCl by 20.8, 27.01, and 11.5% after 6, 12, and 21 days, respectively. Other studies corroborate our results, that while no significant difference was identified, in some instances, chlorophylls a and b concentrations and carotenoids were lower in the saline conditions than CK (11; 33; 53). Other studies corroborated in that while no

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significant difference was identified, in some instances, chlorophylls a and b concentrations and carotenoid were lower in the saline conditions than t control (2; 11; 33; 35; 46; 52; 53). Accordingly, other evidence of reduced chlorophyll and carotenoid contents resulting from salinity was noted in wheat (59; 74; 77), *Salvinia molesta* and *Pistia stratiotes* (79), pea (30; 72), mangrove (51), bean (22; 75), cotton (62), oats (4) and olive saplings (10).

Salinity influences photosynthesis, transpiration rate, reduces stomatal opening, and decreases intercellular carbon dioxide; these influence plant productivity and eventually lead to lower biomass at higher saline concentrations. Both genotypes displayed reduced net photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 at 400 mM NaCl than the CK. This finding can be corroborated by numerous other studies involving other plants (13; 28; 29; 43; 44; 51; 66). Sanchez et al (64) reported significant changes in stomatal conductance and no significant difference in photosynthesis in the quinoa cultivar *Real* when exposed to low salinity (3.85 gL^{-1}) and high salinity (8.05gL^{-1}) over five weeks. Significant differences were observed in the reduction of net photosynthesis (59.39±10.79 to 17.13±2.53 μmol CO_2 $m^{-1}s^{-1}$), transpiration rates (16.05±4.51 to 2.8±0.76 μmol of CO□ m^{-2} s^{-1}), stomatal conductance) (4.84±1.05±to 0.63 μmol H_2O $m^{-1}s^{-1}$, taken as the inverse of stomatal resistance) and intercellular CO_2 (0.60±0.05 to 0.18±0.13 CO_2 μmol mol^{-1}) the quinoa cultivar *Hualhuas* between the CK and 500 mM NaCl after six weeks of treatment (14).

6. CONCLUSION

In conclusion, our results indicated that salinity influences several responses in both *Chadmo* and *Kankolla* towards plant acclimation. The observed effects were attributed to phenotypic plasticity towards salinity. They exhibited significant differences between *Chadmo* and *Kankolla* in shoot/root biomass, membrane stability index, and relative water content, which were significantly lower in *Kankolla*. Similarly, significant differences were observed in the net photosynthesis, stomatal conductance, transpiration rate, and intercellular carbon dioxide. These differences are symptomatic responses to plant salinity. Moreover, as differentially expressed, these traits in both genotypes confirmed that *Chadmo* responded more favorably to salinity than *Kankolla*. Besides, the leaf curling, white tip, and chlorosis in treated *Kankolla* provided further evidence of susceptibility to salinity. This study is important to identify a genotype that is tolerant to salinity. Furthermore, this genotype can be genetically engineered to improve adaptation to the saline environment and bolster agronomic traits and resilience in other crop plants. That is compellingly urgent as more of our arable lands are becoming salinized.

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