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# Biochemical Characterization and Responses of Two Contrasting Genotypes of *Chenopodium quinoa* Willd. to Salinity in a Hydroponic System

**Sirpaul Jaikishun [a,b,c*], Shikui Song [a,b] and Zhenbiao Yang [a,b,d]**

*[a] College of Life Sciences and Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China.*
*[b] FAFU-UCR Joint Center for Horticultural Biology and Metabolomics, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, China.*
*[c] Department of Biology, Faculty of Natural Sciences, University of Guyana, Guyana.*
*[d] Institute of Integrative Genome Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA, USA.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Conceptualization, experimental design, writing of the original draft and preparation were done by authors SJ and ZY. Review and editing and supervision of the laboratory were done by authors ZY and SS. Biostatical analyses and literature research were conducted by authors SJ and SS. All authors read and approved the final manuscript.*

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**Corresponding author: E-mail: sirpaul.jaikishun@uog.edu.gy;*

## ABSTRACT

*Chenopodium quinoa* is a promising species for future food security and combating climate change due to its nutritional content and halophytic nature. This study focuses on the temporal differential responses of the salt-tolerant (*Chadmo*) and the salt-sensitive (*Kankolla*) under control (CK) and 400 mM NaCl arranged under the randomised block designed (RBD). Biochemical features assessed and results indicate a significant difference ($p<0.05$) being identified by ANOVA and Tukey analyses in total chlorophyll (CHL), carotenoids (CAR), proline, glycine betaine (GB), soluble sugars, $K^+$, $Na^+$, $K^+/Na^+$ ratio, $Mg^{2+}$ and $Ca^{2+}$ in both genotypes between the CK and 400 mM NaCl. $Na^+$ increased while $K^+$ and the bivalent ions $Mg^{2+}$ and $Ca^{2+}$ decreased progressively with time points (CK and 24 h) in both genotypes but more pronounced in *Kankolla*. Proline increased by 24.45 and 18.63% between the CK and 24 h after exposure to 400 mM NaCl in *Chadmo* and *Kankolla*, respectively. Similarly, significant increases were observed in ABA, glycine betaine and soluble sugars from the CK to 24 h after exposure to 400 mM NaCl in both genotypes. Using these biochemical 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; biochemical; halophytes; nutrients; proline; quinoa; salinity; salt-tolerant.*

## 1. INTRODUCTION

The global population is advancing towards unprecedented growth amidst the challenges of the effects of climate change influenced by anthropogenic activities. Moreover, demands for food will have to be increased by 70% to provide for ~ 9.8 and 11.2 billion people by the years 2050 and 2100, respectively [1,2]. Quinoa, an ancient crop, has been part of the indigenous inhabitants' diet and culture for over 7000 years [3-5]. In addition to being a halophyte, quinoa has also been noted for its unique adaptations to other abiotic stresses including drought, extreme temperatures, frost, UV-B radiation and growing at varying elevations between 2000 and 4000 m above sea level [6-8]. These adaptive attributes have presumably evolved through its inhabitation in the five uniquely harsh ecotypes stretching across the Andean region: Highlands (Peru and Bolivia), Valleys (Bolivia, Colombia, Ecuador, and Peru), Salares (Bolivia, Chile and Argentina), Yungas (Bolivia) and Lowlands (Chile) [5,9]. The Salares and Highlands areas are predominantly characterised by low precipitation, highly saline soil and frequent frost [10,11]. Interestingly, while some quinoa genotypes have shown a high tolerance level to salinity, a significant difference is observed among the genotypes. Conversely, some genotypes are sensitive to saline and will not germinate and proliferate in saline conditions [12-14]. Quinoa has salt bladders which have more volume space than that of regular epidermal cells and, consequently, can potentially sequester more ions and osmolytes than adjacent cells than plants without salt glands [8,15,16]. This phenomenal adaptation strategically places quinoa in an advantageous position to glycophytes and possibly other halophytes since it can thrive in saline conditions due to its ability to avoid the severe impacts of high salt accumulation [17-19]. According to the United Nations sectorial units on food and health, Food and Agricultural Organization and World Health Organization, quinoa is the only plant food that constitutes and provides all of the essential amino acids required by the human body. It also has a higher protein content than that of rice, barley, corn, rye and sorghum, but similar to that of wheat [20-27]. 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 [12,28,29]. Quinoa can exclude salts and physiologically adjust them to minimize their effects in high concentrations [15,29,30]. Jacobsen et al. [6] observed that the quinoa yield was the highest at 100 to 200 mM NaCl, and thereafter decreased. Further support to this was provided by Hariadi et al. [12], 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. [31] screened 182 quinoa accessions for salt tolerance and found that 25% of them exhibited greater than a 60% germination rate at 250 mM NaCl for seven days. These 15 accessions were further tested in a pot experiment 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. [14] 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. The study endeavours to determine the biochemical responses of two contrasting quinoa genotypes (salt-tolerant *Chadmo* and salt-sensitive *Kankolla*) to 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 [32]. Fresh seeds were germinated in PINDSTRUP substrate (dark sphagnum peat mixed with 30% natural, fibrous material, 50g of micronutrients), and at two true leaf stages (~10 days), the seedlings were transferred into the hydroponic system containing water. After one day, Hoagland solution was added, and 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 [12,33,34]. A consistent level of solution (7L) was maintained by the addition of nutrient solution with the respective NaCl concentrations, as necessary. The seedlings were arranged in a complete randomized block design in the hydroponic treatment box, with three (3) 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 umol $m^{-2}$ $s^{-1}$)/8 h dark cycle. Except for the physiological parameters, stomatal index and epidermal bladder cell measurements which were done on the 45$^{th}$ day, all other samples were harvested immediately at the end of the treatment (400 mM NaCl, 24$^{th}$ 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 Biochemical Analysis

#### (a) Proline content

The fresh plant (24 days old) leaf tissue (50 mg) was homogenized in 5 mL of 3% aqueous sulfosalicylic acid and left for 3 h for extraction to complete. The mixture was then centrifuged at 1500 g for 10 min. An amount of 2 mL of supernatant was added to 2 mL glacial acetic acid and 2 mL acidic ninhydrin. The mixture was boiled at 100 $^{o}$C in a water bath for 60 min, and the reaction stopped abruptly by placing it in an ice bath. An amount of 4 mL toluene was added, thoroughly mixed, and then allowed to warm to room temperature. Reading was done using a spectrophotometer at a wavelength of 520 nm absorbance with toluene as the blank [35].

#### (b) Glycine betaine

Glycine betaine was extracted by grinding leaf (24 days old plant) to a fine powder in liquid $N_2$ (50 mg FW), and 1 mL of methanol (70%) was added. After incubation for 24 h, the homogenate was placed in the ultrasonic apparatus for 30 min. The mixture was then kept at -20$^{o}$C for 12 h and then placed in ultrasonic apparatus for 30 min. The mixture was then centrifuged at 14000 g for 10 min. The supernatant was then extracted and diluted for analysis. Glycine betaine was determined by HPLC, using a cationic exchange column (Dionex Hypersil SCX, 5 µm, 250 × 4.6 mm) at 30°C in isocratic conditions at a flow rate of 1 mL $min^{-1}$. The eluent phase was: sodium phosphate buffer 0.05 m, pH 3.7 (95%), and methanol (5%). Aliquots of the water extract (40 µl) were injected. The eluted glycine betaine was detected by a diode array spectrophotometer set at 195 nm and was characterized and quantified by eluting a standard solution of the pure compound in the same conditions [36].

#### (c) Abscisic Acid (ABA)

Fresh leaf tissue (24 days old plant) sample was ground with mortar and pestle to powder in liquid nitrogen. An amount of 100 mg (0.05 g) was then dissolved in 900 µl methanol (70%, v/v), to which 100 µl internal standard ABA (i-ABA) was added and placed in an ultrasonic water bath for ½ h and then left overnight in -20 $^{o}$C. The sample was then placed in an ultrasonic water bath for ½ h, and extracts were centrifuged at 14,000 g for 10 minutes at 4$^{o}$C. The supernatant was collected to which 500 µl methanol (70%, v/v). After precipitation, it was placed in an ultrasonic bath for ½ h and the supernatant was extracted. The supernatant was removed in a SpeedVac to ~300 µl. An amount of 700 µl 1% formic acid (v/v) was then added and vortexed for 1 min and thereafter placed at -20$^{o}$C for 3 h. Solid-phase extraction (SPE, Oasis MCX (mixed-mode cation exchange) extraction cartridge, 60mg 3mL). The

cartridge was then activated (2 mL 70% methanol, 2 mL 0.1M HCl, 2 mL 1% formic acid.) and samples were loaded and flushed with 2 mL 1% formic acid [37].

**(d) Soluble sugars (glucose, fructose, sucrose and lactose)**

Samples (24 days old plant) were harvested and dried at 85 $^{o}$C for 48 h and were then finely ground. An amount of 30 mg powdered sample was then placed in a 2 mL centrifugal tube. To this, 500 µl solvent (methanol: water, 3:1) and 30 µl vanillic acid (5 mg/mL). The mixture was then ultrasonic water bath for 30 minutes and then centrifuged at 12000 g for 10 minutes at room temperature. Maximum supernatant was then transferred to 1.5 mL tubes and then centrifuged at 12000 g for 10 minutes at room temperature. An amount of 150 µl supernatant was then transferred to a 2 mL sample vial and placed SpeedVac (Thermo Fisher) vacuum rotary evaporation for 4 h. Methoxypyridine solution {methoxyamine hydrochloride (42 mg) + pyrimidine (2.1 mL)}2(80 µl, 20 mgmL$^{-1}$) was then added to the dried samples for derivatization and gasification then placed in an oven at 80 $^{o}$C for 20 min. An amount of 80 µl BSTFA (N,O-Bis{trimethylsilyl}trifluoroacetamide) + 1% TMCS (trimethylchlorosilane) mixture was then added in the derived solution then placed in an oven at 70$^{o}$C for 1 h. Extract (~0.2 mL) was then micro-filtered and then subjected to non-targeted metabolites in the instrument (LECO PegasusHT GC-Q ToF MS - Capillary tube: Type code: DB-5 MS, Size: 30 m × 0.25 mm × 0.25 µm. Injection temp: 280$^{o}$C, capillary temp: 275 $^{o}$C, split ratio: 10:1, flow rate: 1.5 mLmin$^{-1}$) for the analysis of the sugars [38,39].

**(e) Cationic measurement (sodium, potassium, magnesium and calcium)**

After preparation and digestion of the leaf sample (100 mg DW), sodium ($Na^{+}$) and potassium ($K^{+}$) were estimated through flame photometry [40] while magnesium ($Mg^{2+}$) and calcium ($Ca^{2+}$) were quantified through flame atomic absorption spectrometry [40-42].

## 2.3 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 post-hoc 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 analyses.

# 3 RESULTS

## 3.1 Proline, ABA and glycine betaine

Consistent increases in proline content were observed in both the *Chadmo* and *Kankolla* genotypes, except for *Kankolla* between CK and 0.5 h. After 0.5 h *Chadmo* increased from 10.21±1.7 to 11.04±1.32 µmolg$^{-1}$ while *Kankolla* decreased from 7.38±0.82 to 6.92±0.65 µmolg$^{-1}$. The highest proline content in the *Kankolla* was observed at 24 h at 10.08±1.31 µmolg$^{-1}$ while for the *Chadmo* it was 12.83±0.32 µmolg$^{-1}$ at 24 h (Fig. 1 (a)). Between the CK (0h) and 24 h, *Chadmo* increased by 20.45% while *Kankolla* increased by 18.63%. No significant difference was observed in *Chadmo* and *Kankolla* proline contents across the different time points at $p<0.05$.

ABA content was higher in the salt-sensitive *Kankolla* in CK with 1.95±0.34 µmolg$^{-1}$ and the *Chadmo* with 1.02.±0.04 µmolg$^{-1}$, which represented a significant difference at $p<0.05$. At 0.5 h, the amount of ABA was similar in the salt-sensitive *Kankolla* and salt-tolerant *Chadmo*. After that, ABA increased but significantly at 6 h and 24 h in the salt-tolerant *Chadmo* with 3.56±0.8 and 3.19±0.95 µmolg$^{-1}$ and remained more consistent with the salt-sensitive *Kankolla* at 1.59±0.59 and 1.49±0.07 µmolg$^{-1}$, respectively. The highest ABA content in salt-tolerant *Chadmo* was observed at 6 h (3.56±0.8 µmolg$^{-1}$) and 24 h (3.19±0.95 µmolg$^{-1}$) while for salt-sensitive *Kankolla*, it was at the CK (1.95±0.34 µmolg$^{-1}$) (Fig. 1(b)). Contrastingly, however, between the CK (0 h) and 24 h ABA decreased significantly while *Chadmo* ABA increased significantly.

A similar trend was observed with glycine betaine in both varieties at the control and treatment. The salt-tolerant *Chadmo* and salt-sensitive *Kankolla* varieties had 1.08 and 0.61 µgg$^{-1}$ (DW) at the control which increased by 13 and 22% respectively, after ½ h. After that, salt-tolerant *Chadmo* decreased at 1 and 3 h by 14 and 33%,

respectively, while the salt-sensitive *Kankolla* decreased steeply at 3 h (Fig. 2). Analysis of variance identified significant differences ($p<0.05$) among the time points of the salt-tolerant *Chadmo* varieties only, and no significant difference was observed between the control and 24 h in both varieties.

## 3.2 Soluble Sugars

The soluble sugars (fructose, glucose, sucrose, and lactose) levels showed an increasing trend with increasing salinity relative to the CK except for sucrose in *Chadmo*. However, this increase was not significant, except for lactose in *Chadmo* and glucose, sucrose and lactose in *Kankolla*. For *Chadmo*, lactose increased between the CK and 24 h from 0.008±0.000 to 0.029±0.005 $mgg^{-1}$ (DW)and 0.279±0.105 $mgg^{-1}$ (DW) between the CK and 24 h while in *Kankolla*, glucose, sucrose and lactose increase from 0.094±0.012 $mgg^{-1}$ (DW) to 0.419±0.078 $mgg^{-1}$ (DW), 0.24±0.012 to 0.412±0.095 $mgg^{-1}$ (DW) and 0.241±0.067 to 0.845±0.106 $mgg^{-1}$ (DW), respectively. Interestingly, the only decrease in the soluble sugars was observed with lactose from 0.279±0.105 to 0.084±0.01 $mgg^{-1}$ (DW) between CK and 24 h in *Chadmo* (Table 1).

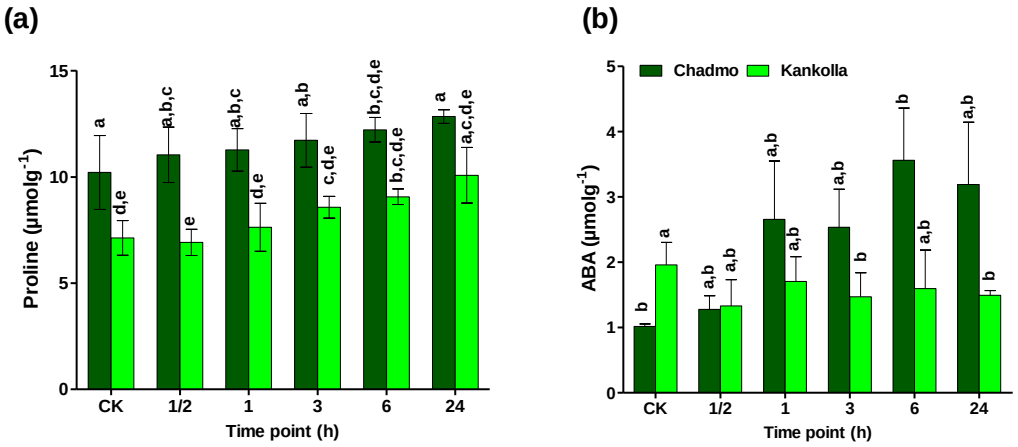


**Fig. 1. Response of *Kankolla* and *Chadmo* to salinity treatment at the different time points in (a) proline content and (b) ABA content. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*, respectively**

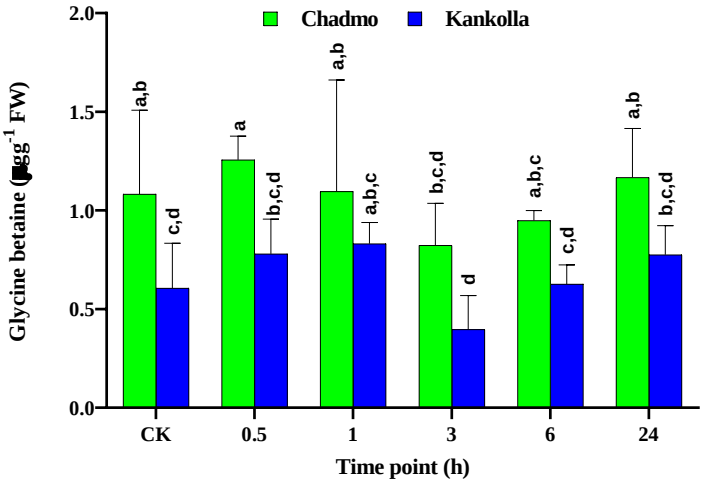


**Fig. 2. Response of *Kankolla* and *Chadmo* in glycine betaine content to CK and 400 mM NaCl salinity treatment at different time points. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla* at different time points, respectively**

**Table 1. Response of *Kankolla* and *Chadmo* to CK and 400 mM NaCl at different time points in some soluble sugar content. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 (Tukey pairwise analyses) in *Chadmo* and *Kankolla*, respectively**

| Sugar ($mgg^{-1}$ DW) | Genotype | Post-treatment time point (h) | | | | | |
|---|---|---|---|---|---|---|---|
| | | CK | 0.5 | 1 | 3 | 6 | 24 |
| Fructose | *Chadmo* | 3.455±1.251*a* | 3.012±0.902*a* | 3.729±0.741*a* | 3.707±0.583*a* | 3.931±0.339*a* | 4.324±0.457*a* |
| | *Kankolla* | 3.004±0.457*a* | 2.960±0.864*a* | 2.922±0.946*a* | 3.323±0.681*a* | 3.643±0.514*a* | 3.978±0.284*a* |
| Glucose | *Chadmo* | 0.427±0.091*a* | 0.475±0.085*a* | 0.411±0.095*a* | 0.464±0.005*a* | 0.495±0.101*a* | 0.529±0.186*a* |
| | *Kankolla* | 0.094±0.012*b* | 0.113±0.081*b* | 0.103±0.010*b* | 0.344±0.067*a* | 0.472±0.051*a* | 0.419±0.078*a* |
| Sucrose | *Chadmo* | 0.008±0.000*b* | 0.008±0.001*b* | 0.007±0.002*b* | 0.005±0.000*b* | 0.005±0.001*b* | 0.029±0.005*a* |
| | *Kankolla* | 0.24±0.012*b* | 0.239±0.091*a* | 0.313±0.071*a* | 0.362±0.059*a* | 0.393±0.097*a* | 0.412±0.095*a* |
| Lactose | *Chadmo* | 0.279±0.105*a* | 0.036±0.006*b* | 0.016±0.006*b* | 0.057±0.009*b* | 0.076±0.005*b* | 0.084±0.011*b* |
| | *Kankolla* | 0.241±0.067*c* | 0.249±0.047*c* | 0.266±0.046*c* | 0.525±0.142*b* | 0.755±0.049*a,b* | 0.845±0.106*a* |

### 3.3 Potassium ($K^+$), Sodium ($Na^+$), Magnesium ($Mg^{2+}$) and Calcium ($Ca^{2+}$)

Leaf $Na^+$ content was similar in both the varieties at the control but significantly increased when exposed to salinity. Salt-tolerant *Chadmo* and salt-sensitive *Kankolla* at the control was 0.67 and 0.60%, but this increased significantly ($p<0.05$) under salinity to 27.28 and 20.36%, respectively. The amount of $Na^+$ accumulated in the leaf of salt-tolerant *Chadmo* was 7% more than salt-sensitive *Kankolla*, representing a significant difference ($p<0.05$) between them under salinity. Leaf $K^+$, on the contrary, was slightly higher (0.87%) in salt-tolerant *Chadmo* than salt-sensitive *Kankolla* in control while being exposed to the saline condition $K^+$ increased to 1.27% with 4.27 and 3%, respectively (Fig. 3 (b)). Leaf $Na^+$ indicated consistent increases with the time points while, on the contrary, $K^+$ showed a consistent decrease with the time points in both *Chadmo* and *Kankolla* (Fig. 3 (a)). The $K^+/Na^+$ ratio was significantly different between the CK and the different time points for both varieties.

Both $Mg^{2+}$ and $Ca^{2+}$ decreased at higher salt concentrations and with increasing exposure time to salinity as compared to the CK in both genotypes. $Mg^{2+}$ decreased consistently with increasing exposure time to salinity as compared with the CK for both genotypes. For *Chadmo*, the decrease from the CK to 24 h was from 25.76±2.42 to 10.51±1.68% representing a 59.2% reduction (Fig. 4 (a)). The most notable decrease was observed between the CK and ½ h which was from 25.76±2.41 to 19.98±5.73% representing a 22% reduction while the least reduction was from 6 to 24 h (11.22±2.54 to 10.51±1.68%) representing a 6.49 % reduction. For *Kankolla*, $Mg^{2+}$ decreased by 64% from the CK to 24 h, 23.22±1.36 to 8.22±1.01%, respectively. The highest decrease for $Mg^{2+}$ in *Kankolla* was observed from 6 to 24 h, which was from 11.62±1.55 to 8.22±1.01%, which represents a 29% reduction (Fig. 4 (a)).

The decrease of $Ca^{2+}$ from the CK to 24 h was 32.61±3.47 to 9.60±1.58% and 36.78±1.16 to 13.01±2.16% representing 70.55 and 64.64% in *Chadmo* and *Kankolla*, respectively. In *Chadmo*, the highest decrease at the successive time point was observed from 1 to 3 h which reduced from 28.75±3.73 to 12.34±1.21% representing a 57.16% reduction while at the subsequent time point, the decrease was negligible (Fig. 4 (b)). For *Kankolla*, as with *Chadmo*, $Ca^{2+}$ decreased with the increasing exposure time to salinity. The highest decrease from 24.88±1.46 to 13.65±1.28% representing a 45.11% reduction was observed from the time point 1 to 3 h. A significant difference was observed between the CK and time points and differential significant differences among the time points for both genotypes at $p<0.05$.

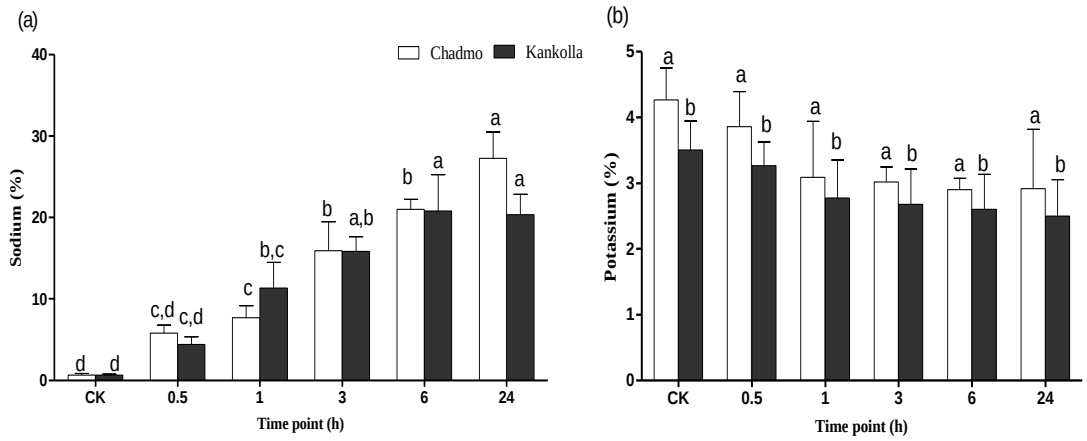


**Fig. 3. Response to salinity at the CK and 400 mM NaCl at different time points (a) sodium and (b) potassium. Means ± SD (n=3). Different letters indicate a significant difference at $p<0.05$ in *Chadmo* and *Kankolla*, respectively**

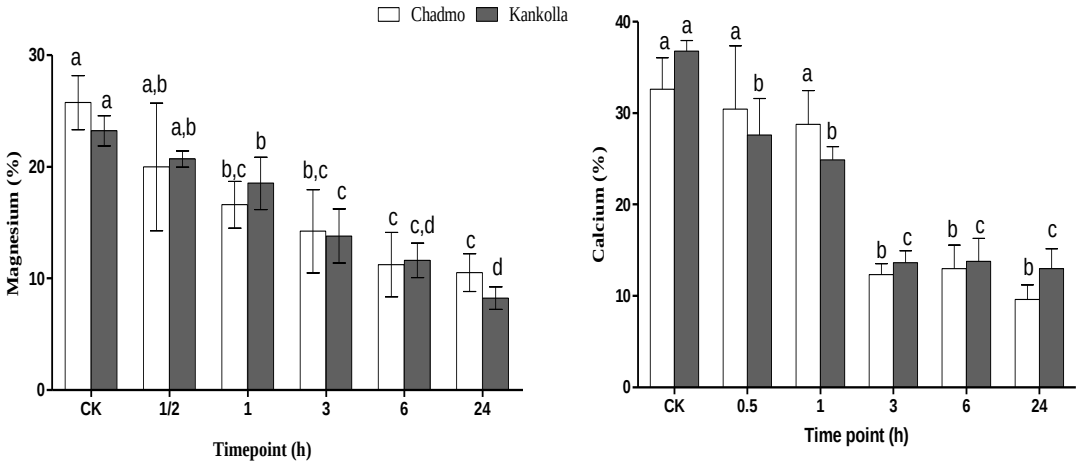


**Fig. 4. Response to salinity in magnesium and calcium contents at the CK and different time points. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*, respectively**

## 4. DISCUSSION

Chlorophyll and carotenoid contents decreased more at 400 NaCl than in the CK for both *Chadmo* and *Kankolla*. Likewise, the reduction between the control and 400 mM NaCl in salt-sensitive *Chadmo* was not significant 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 [28,43-47]. Ruffino et al. [48] 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 as compared with the CK. They posited that Chlorophyll b had a significant difference while 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 with 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 [44,46,47]. Other studies corroborated in that while no significant difference was identified, in some instances, chlorophylls a and b concentrations and carotenoids were lower in the saline conditions than t control [43,44,46,47,49-51]. Accordingly, other evidence of reduced chlorophyll and carotenoid contents resulting from salinity was noted in wheat [42,52,53], *Salvinia molesta* and *Pistia stratiotes* [54], pea [55,56], mangrove [57], bean [58,59], cotton [60], oats [34] and olive saplings [61].

For proline, glycine betaine, ABA, and soluble sugars, they all indicated relative increases in both the genotypes and more so, at 400 mM NaCl and with increasing exposure time to salinity. Furthermore, this increase in these osmolytes was more pronounced in *Chadmo* than in *Kankolla* giving more credence to *Kankolla* being more sensitive to salinity. Accumulation of proline and glycine betaine due to abiotic stresses has been well-researched in many plant species [62-66]. Shabala and Mackay [67] stated that proline serves as an important osmolyte that protects the cells from toxic levels of $Na^+$. Moreover, epidermal bladder cells have high levels of proline transporter further indicating its functional role in the uptake of proline from neighbouring cells [16]. Accumulated glycine betaine in the chloroplast is also symbolic of smothering the effects of salt, hence reducing its impacts on photosynthesis [65,68-74]. In support, Ruffino et al. [48] exposed seedlings of the quinoa cultivar *Sajama* to 250 mM NaCl and asserted that after 6, 12 and 21 days, proline content increased by 23.8, 46.2 and 85.6% respectively as compared with the CK in a hydroponic system. Vasile et al. [75] assessed the effect of salinity on proline in three cultivars

of quinoa during germination, *Titicaca*, *Puno,* and *Vikings* and concluded it increased by 64.41, 30.86 and 153.19% respectively between the CK and 300 mM NaCl. Subjecting four Chilean genotypes to 300 mM NaCl over 15 days, proline increased by three-to-five folds than the CK [76] while Orsini et al. [28] alluded to a ten-fold increase in *BO78* between 600 and 750 mM NaCl relative to the CK. Moreover, consistent increases were observed between the CK and the NaCl concentrations (50, 100, 200 and 300 mM NaCl) in the proline content of the seeds after 5 days of germination. Regarding glycine betaine, increases were observed in *Sajamo* cotyledons when exposed to 250 mM NaCl, after 6, 12 and 21 days glycine betaine increased by 18.18, 17.74, and 38.55% respectively as compared to the CK. Similarly, Ruiz et al. [77] studied the effects of salinity (300 mM NaCl) on ABA in the quinoa landraces *R49* and *Villarica* seedlings and concluded that ABA levels increased significantly in treated shoots as compared with the CK. Significantly, ABA increased from 0.52 to 2.11 nmol $g^{-1}$ DW representing a 305.76% hike in *R49* and from 0.63 to 1.85 nmol $g^{-1}$ DW representing a 193.65% increase in *Villarica* between the CK and 300 mM NaCl treatment after 120 h exposure.

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 [28,42-46]. The low $K^+$ content in the leaves is indicative of the effect of salinity on its competitive absorption from nutrients or repulsive response due to the same ionic charge. Toxic levels of sodium and chloride ions also create a significant ionic imbalance in plants [30,49,67,78-80]. Ruffino et al. [48] studied the response of quinoa cultivar *Sajama* to 250 mM NaCl for $K^+$ and $Na^+$ in which they postulated an increase in $Na^+$ by 22.4, 22.5 and 24.1% after 6, 12 and 21 days of salinity exposure as compared with the CK. On the contrary, $K^+$ decreased by 5.9, 7.4 and 5.1% over the same period when compared with the CK in 250 mM NaCl. These results can be corroborated by Hariadi et al. [12] with the quinoa *cv 5206* in which the plants were exposed to salinity (100 – 500 mM NaCl) after 70 days and concluded that $Na^+$ increased gradually in the leaf sap. The differences in the $Na^+$ and $K^+$ between the time points and CK inferred a higher $K^+/Na^+$ ratio for both salt-tolerant *Chadmo* and salt-sensitive *Kankolla*. An increase in $K^+/Na^+$ in plant leaves characterizes higher levels of tolerance to salinity in both halophytes and glycophytes [30,81]. Higher $K^+/Na^+$ was observed in the quinoa cultivar *cv 3706* after being exposed to 400 mM NaCl as compared with the CK. Reduction in $K^+$ will inevitably affect plant metabolism since its presence activates several enzymes, including rubisco which enhances the biosynthesis of chlorophyll. Hence, the increased $K^+/Na^+$ ratio due to the increase in $Na^+$ will lead to a decline in the photosynthetic potential of the plant [13,82-84]. In support, Adolf et al. [13] studied *Titicaca* and *Utsusaya* quinoa varieties and concluded that both indicated significant increases in $K^+$ and $Na^+$ in the leaf of salt in treated plants as compared to the control. $Ca^{2+}$ does play an integral role in salt tolerance in plants by reducing the toxicity of NaCl [85]. $Ca^{2+}$ increase is triggered by low $K^+$ or elevated levels of $Na^+$ in the tissue which will then promote stress signalling pathways to enhance salt tolerance [12,86]. In support, Orsini et al. [28] exposed *BO78* (quinoa variety) to salinity from 150 to 750 mM NaCl and observed an increase in $Mg^{2+}$ content with increasing salt concentration. Increasing $Mg^{2+}$ is also critical because of its role in metabolism and chlorophyll. More so, $Na^+$, $K^+$ and $Ca^{2+}$ all have intricate networks of interplay and are collaboratively involved in salt tolerance in plants [30,85,87,88]. $Mg^{2+}$ was also affected by salinity as decreased significantly in both genotypes (*Chadmo* and *Kankolla*) with increasing exposure period from ½ to 24 h in 400 mM NaCl as compared to the CK. Increasing $Mg^{2+}$ is also critical because of its role in metabolism and chlorophyll synthesis [28]. The decrease in $Mg^{2+}$ in *Kankolla* and its functional role in chlorophyll biosynthesis may be the reason for chlorosis in its leaves.

## 5. CONCLUSION

In conclusion, significant differences were observed in the net photosynthesis, increases in soluble sugars, glycine betaine, proline and ABA are also osmolyte-response to salinity to enhance physiological functions and smother the effects of salinity. Moreover, the increases in Ca and Na ions and associated decreases in K and Mg ions to serve to reduce the ionic toxicity in cells were observed in both *Chadmo* and *Kankolla* and but more significantly in the latter. Furthermore, the leaf curling, white tip and chlorosis in treated *Kankolla* provided further evidence of susceptibility to salinity. The results of this study are important as they identify a genotype that is tolerant to salinity and hence

can be genetically engineered to improve adaptation to the saline environment, bolster agronomic traits and resilience in other crop plants, which is compellingly urgent as more of our arable lands are becoming salinized.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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