Biochemicalcharacterization oftwo contrasting genotypes of *Chenopodium quinoa*Willd.to salinity in a hydroponic system at the seedling stage

Abstract: *Chenopodium quinoa* is a promising species for future food security and combating climate change due to its nutritional content and halophyticnature. This study focuses on the temporal differential responses of the salt-tolerant (*Chadmo*) and the salt-sensitive (*Kankolla*) under control (CK) and 400 mMNaCl. Biochemical features assessed and results indicatea significant difference ($p<0.05$) being identified by ANOVA and Tukey analyses intotal chlorophyll (CHL), carotenoids (CAR), proline, glycinebetaine (GB), soluble sugars, K^+, Na^+, K^+/Na^+ ratio, Mg^{2+} and Ca^{2+} in both genotypes between the CK and 400 mMNaCl. 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 exposureto 400 mMNaClin *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 mMNaCl in both genotypes. Using these biochemical responses to salinity, *Chadmo* proved to be the better-performing genotype when exposed to 400 mMNaCl 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 advancingtowards unprecedented growth amidst the challenges of the effects of climate change influenced by anthropogenicactivities. 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(FAO et al 2018, Wang et al 2013). Quinoa, an ancient crop, has beenpart of the indigenous inhabitants' diet and culture for over 7000 years(Jarvis et al 2017, Mujica & Jacobsen 2006, Valencia-Chamorro 2003). 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 radiationand growing at varying elevations between 2000 and 4000 m above sea level (Jacobsen et al 2003, Ruiz et al 2016, Shabala et al 2012). 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) (Bazile et

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al 2016, Valencia-Chamorro 2003). The Salares and Highlands areas are predominantly characterised by low precipitation, highly saline soil and frequent frost (Jacobsen et al 2007, Prado et al 2000). 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 (Adolf et al 2012, Hariadi et al 2010, Morales et al 2011). Quinoa has salt bladders which have more volume space than that of regular epidermal cells and, consequently, can potentiallysequester more ions and osmolytes than adjacent cells than plants without salt glands(Blumwald 2000, Brownlee 2018, Shabala et al 2012). 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 (Hasegawa 2013, Munns 2002, Riccardi et al 2014). 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 (Hariadi et al 2010, Orsini et al 2011, Pulvento et al 2012). Quinoa can exclude salts and physiologically adjust them to minimize their effects in high concentrations (Blumwald 2000, Flowers & Colmer 2008, Pulvento et al 2012).Jacobsen et al (2003)observed that the quinoa yield was the highest at 100 to 200 mMNaCl, and thereafter decreased. Further support to this was provided by Hariadi et al (2010), recorded significant inhibitory effects on seed germination at concentrations higher than 400 mMNaCl, while optimal plant growth was obtained between 100 and 200 mMNaCl with *Titicaca* over a 70-day growth period. Gómez-Pando et al (2010)screened 182 quinoa accessions for salt tolerance and found that 25% of them exhibited greater than a 60% germination rate at 250 mMNaCl for seven days. These 15 accessions were further tested in a pot experiment at 300 and 340 mMNaCl. 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 (2011) also observed that at 300 mMNaCl, quinoa cultivars *Chipaya* and *Ollague* showed a decrease in fresh weight but at 450 mMNaCl they sustained 50 and 40% higher transpiration rate than that of the CK, respectively.The study endeavours to determine thebiochemicalresponses of two contrasting quinoa genotypes (salt-tolerant *Chadmo* and salt-sensitive *Kankolla*) to salinity under the hydroponic system.

Comment [MS1]: An economic importance of crop should be emphasized

Comment [MS2]: Area under cultivation, total production and productivity should also be mentioned.

Comment [MS3]: Annual yield losses due to abiotic stresses such as drought, temperature, salinity should be mentioned

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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. TheUnited States Department of Agriculture (USDA) in Washington, USA kindly provided the seeds(USDA & NRCS. 2018). Fresh seeds were germinated in PINDSTRUP substrate(dark sphagnum peat mixed with 30% natural, fibrous material, 50g of micronutrients), and at two true leaf stage (~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 mMNaCl day^{-1}) to avoid osmotic shock and damage to root until the 400 mMNaCl concentration threshold of treatment was achieved (Bai et al 2018, Hariadi et al 2010, Hoagland & Arnon 1950). Consistent level of solution (7L) was maintained by the addition of nutrient solution with the respective NaCl concentrations, as necessary. 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 umol m^{-2} s^{-1})/8 h dark cycle. Except forthe 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 mMNaCl, 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).

Comment [MS4]: Name of the genotype should be written in italics

2.2 BIOCHEMICAL ANALYSIS

2.2 (a) Proline content

The fresh plant (24 days old) the fresh 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 at 520 nm, using toluene as the blank (Bates et al 1973).

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2.2 (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 in -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 (DionexHypersil 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%), 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(Di Martino et al 2003, Kikuchi et al 1993).

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2.2 (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 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 (Almeida Trapp et al 2014, Robertson 1987).

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

Samples (24 days old plant) were harvested and dried at 85 ^{o}Cfor 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 µlvanillic 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

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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 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 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 derived solution then placed in oven at 70 ^{o}C for 1 h. Extract (~0.2 mL) was then micro-filtered and then subjected for 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(Fiehn 2016, Haliński & Stepnowski 2013).

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

After preparation and digestion of leaf sample (100 mg DW),sodium (Na^{+}) and potassium (K^{+}) were estimated through flamephotometry (Tandon 2005) while magnesium (Mg^{2+}) and calcium (Ca^{2+}) were quantified through flame atomic absorption spectrometry(Peronico & Raposo 2016, Sairam et al 2002, Tandon 2005).

2.3DATA 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 (a) 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

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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 (Figure 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*prolinecontents across the different time pointsat $p<0.05$.

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}) (Figure 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 treated. 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 (Figure 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 the varieties.

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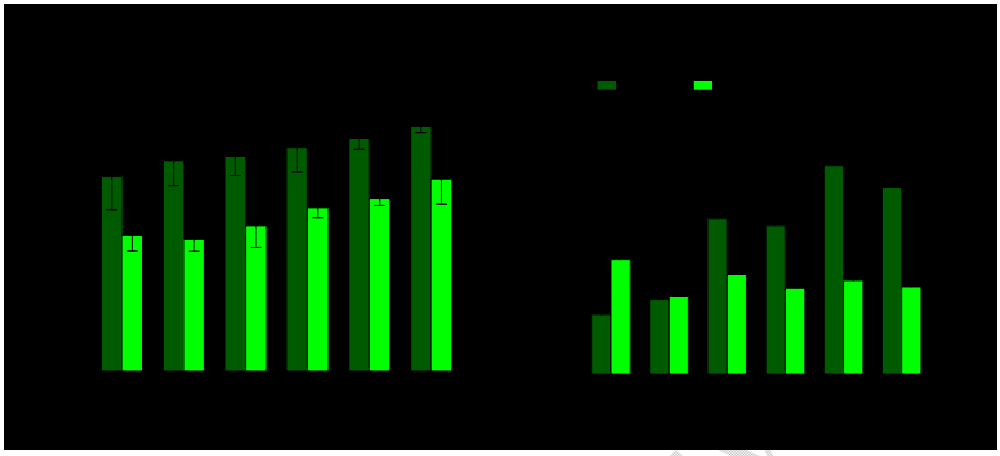

Figure 1 Response of *Kankolla* and *Chadmo* to salinity treatment at the different timepoints in (a) proline content (b) ABA content. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*, respectively

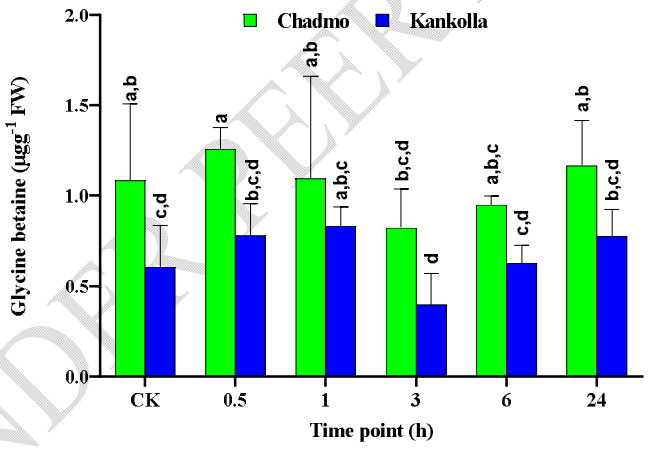

Figure 2Response of *Kankolla* and *Chadmo* in glycine betaine content to CK and 400mMNaCl salinity treatment at different time points. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* and *Kankolla*at different time points, respectively

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3.8 (b) Soluble sugars

The soluble sugars (fructose, glucose, sucrose, and lactose) levels showed an increasing trend with increasing salinity relative to the CKexcept 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)and0.279±0.105mgg^{-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).

Table 1 Response of *Kankolla* and *Chadmo* to CK and 400 mMNaCl 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±0.90***2a***	3.729±0.741***a***	3.707±0.58***3a***	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±0.10***6a***

3.8 (c) 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 in 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 (Figure 3 (b)). Leaf Na^+ indicated consistent increases with the timepoints while, on the contrary, K^+showed a consistent decrease with the time points in both *Chadmo* and *Kankolla* (Figure 3 (a)). The K^+/Na^+ ratio was significantly different between the CK and the different time points for both varieties.

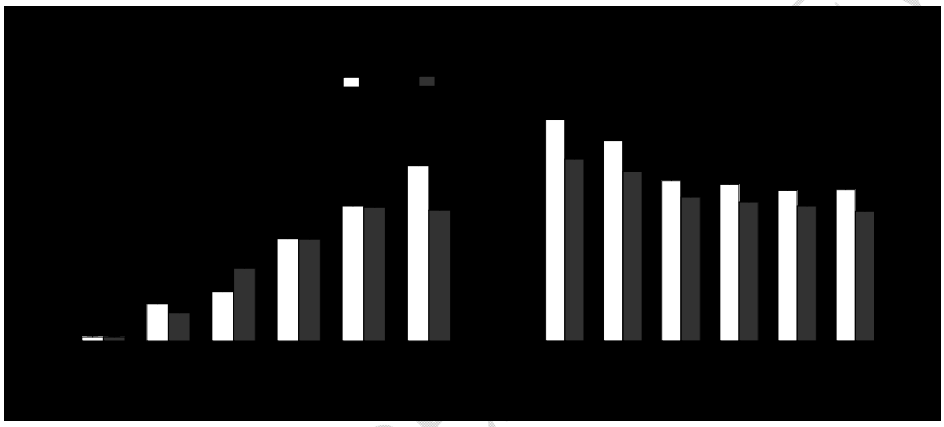

Figure 3Response to salinity at the CK and 400 mMNaCl atdifferent time points (a) sodium and (b) potassium. Means ± SD (n=3). Different letters indicate a significant difference at $p<0.05$ in *Chadmo* and *Kankolla*, respectively.

Both Mg^{2+} and Ca^{2+}decreased at higher salt concentration and with increasing exposure time to salinity as compared to the CK in both the 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 59.2% reduction (Figure 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 (Figure 4 (a)).

The decreaseof $Ca^{2+}$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 (Figure 4 (b)). For *Kankolla*, as with *Chadmo*, Ca^{2+} decreased with the increasing exposure time to salinity. The highest decreased 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 $p<0.05$.

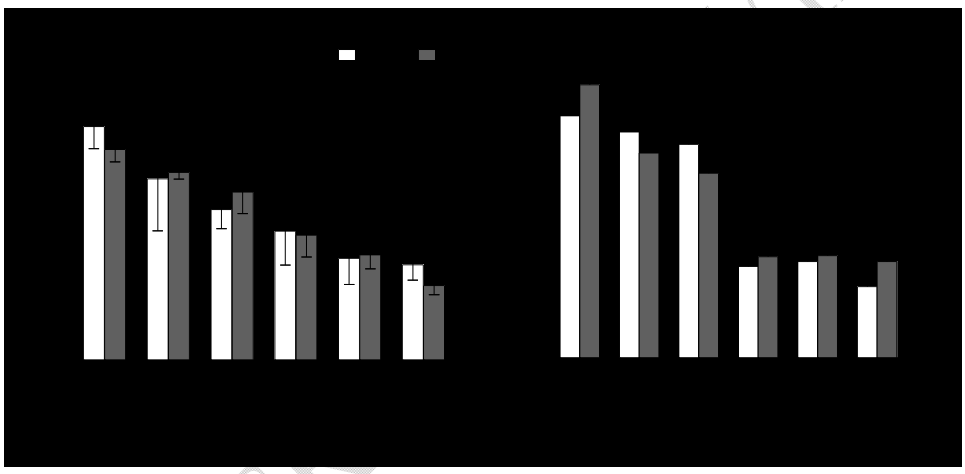

Figure 4Response to salinity in magnesium and calcium contents at the CK and different time points. Means ± SD (n=3). Different letters indicate a significant difference at p<0.05 in *Chadmo* **and** *Kankolla***, respectively**

DISCUSSION

Chlorophyll and carotenoid contents decreased more at 400 NaCl than in the CK. Likewise, the reduction between the control and 400 mMNaCl 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*, provided further evidence that salinity impacts the biosynthesis of

chlorophyll or is destructive post-synthesis (Aghaleh et al 2009, Chaves et al 2009, Gomes et al 2017, Houimli et al 2010, Orsini et al 2011, Pinheiro et al 2008). Ruffino et al (2010)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 mMNaCl 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 mMNaCl. Similarly, carotenoid contents in *Sajama* decreased between the CK and 250 mMNaCl 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 (Chaves et al 2009, Houimli et al 2010, Pinheiro et al 2008). Other studies corroborated in that while no significant difference was identified, in some instances, chlorophylls a and b concentrations and carotenoid were lower in the saline conditions than t control (Aghaleh et al 2009, Chaves et al 2009, Houimli et al 2010, Jampeetong & Brix 2009, Netondo et al 2004, Perez-Romero et al 2019, Pinheiro et al 2008). Accordingly, other evidence of reduced chlorophyll and carotenoid contents resulting from salinity were noted in wheat (Sairam et al 2002, Srivastava et al 1988, Thind & Malik 1988), *Salviniamolesta* and *Pistiastratiotes*(Upadhyay & Panda 2005), pea (Hernandez et al 1995, Shahid et al 2012), mangrove (Parida et al 2004), bean (Gadallah 1999, Taibi et al 2016), cotton (Saleh 2012), oats (Bai et al 2018) and olive saplings (Centritto et al 2003).

For proline, glycinebetaine, ABA, and soluble sugars, they all indicated relative increases in both thegenotypes and more so, at 400 mMNaCl and with increasing exposure time to salinity. Furthermore, this increase in these osmolytes was more pronounced *Chadmo* than *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 (Bassi & Sharma 1993, Munns & Rawson 1999, Naidu et al 1991, Rhodes et al 2002, Saxena et al 2010). Shabala and Mackay (2011)stated that proline serves as important osmolytes that protect 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 (Brownlee 2018). Accumulated glycine betainein the chloroplast also symbolic in smothering the

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effects of salt, hence reducing its impacts on photosynthesis (Genard 1991, Hayat et al 2012, Kishor et al 2015, Rhodes et al 2002, Robinson & Jones 1986, Schmidt et al 2007, Sharma & Dietz 2006, Yang et al 2003). In support, Ruffino et al (2010) exposed seedlings of the quinoa cultivar *Sajama* to 250 mMNaCl 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 (2019)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 mMNaCl. Subjecting four Chilean genotypes to 300 mMNaCl over 15 days, proline increased by three-to-five folds than the CK (Ruiz-Carrasco et al 2011) while Orsini et al (2011) alluded to a ten-fold increase in *BO78* between 600 and 750 mMNaCl relative to the CK. Moreover, consistent increases were observed between the CK and the NaCl concentrations (50, 100, 200 and 300 mMNaCl) 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 mMNaCl, 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 (2017) studied the effects of salinity (300 mMNaCl) on ABA in the quinoa landraces *R49* and *Villarica* seedlings and concluded that ABA levels increased significantly 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 mMNaCl treatment after 120 h exposure.

Leaf chlorosis, white spots/appearance and leaf rolling were also observed in treated salt-sensitive *Kankolla*, provided further evidence that salinity impacts the biosynthesis of chlorophyll or is destructive post-synthesis (Aghaleh et al 2009, Chaves et al 2009, Gomes et al 2017, Houimli et al 2010, Orsini et al 2011, Sairam et al 2002). The low K^+ content in the leaves is indicative of the effect of salinity on its competitive absorption from nutrient or repulsive response due to the same ionic charge. Toxic levels of sodium and chloride ions also create a significant ionic imbalance in plants(Flowers & Colmer 2008, Jampeetong & Brix 2009, Niu et al 1995, Shabala & Mackay 2011, Sharma et al 2005, Xu et al 2009).Ruffino et al (2010) studied the response of quinoa cultivar *Sajama* to 250 mMNaCl 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

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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 mMNaCl. These results can be corroborated by Hariadi et al (2010) with the quinoa *cv 5206* in which the plants were exposed to salinity (100 – 500 mMNaCl) 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*. Increase in K^+/Na^+ in plant leaves characterizes higher levels of tolerance to salinity in both halophytes and glycophytes(Flowers & Colmer 2008, Shabala & Cuin 2008). Higher K^+/Na^+ was observed in the quinoa cultivar *cv 3706* after being exposed to 400 mMNaCl 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 (Adolf et al 2012, Marschner 1995, Shabala 2003, Shabala 2013). In support, Adolf et al (2012) 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(Shabala et al 2006). Ca^{2+} increase is triggered by low K^+ or elevated level of Na^+ in the tissue which will then promote stress signalling pathways to enhance salt tolerance(Hariadi et al 2010, Luan et al 2009). In support, Orsini et al (2011) exposed *BO78* (quinoa variety) to salinity from 150 to 750 mMNaCl and observed an increase in Mg^{2+} content with increasing salt concentration. Increasing Mg^{2+} also critical because of its role in metabolism and chlorophyll.More so, Na^+, K^+ and Ca^{2+} all haveintricate networks of interplay and collaboratively involved in salt tolerance in plants(Demidchik et al 2010, Flowers & Colmer 2008, Munns & Tester 2008, Shabala et al 2006). $Mg^{2+}$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 mMNaCl as compared to the CK. Increasing Mg^{2+} also critical because of its role in metabolism and chlorophyll synthesis (Orsini et al 2011). The decrease in Mg^{2+} in *Kankolla* and its functional role in chlorophyll biosynthesis may be the reason for chlorosis in its leaves.

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 smothering the effects of salinity.Moreover, the increases in

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Ca and Na ions andassociateddecreases in K and Mg ions to serve to reduce the ionic toxicity in cellswere 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 improving 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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