Australian Journal of Crop Science AJCS
AJCS 6(2):357-368 (2012) ISSN:1835-2707

Effect of NaCl salinity on water relations, photosynthesis and chemical composition of Quinoa (*Chenopodium quinoa* Willd.) as a potential cash crop halophyte

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Abstract

Despite the large interest in the use of *Chenopodium quinoa* as a crop on extreme habitats, very little is known about growth response and seed yield under saline conditions. As a prerequisite for its sustainable utilization in salt-affected areas, this study aimed to unravel individual physiological and morphological mechanisms that determine its salt tolerance. Hence, the plants were grown in a hydroponic quick check system with 0, 100, 200, 300, 400, and 500 mM NaCl (equivalent to 0, 20, 40, 60, 80 and 100% seawater salinity). Growth of *C. quinoa* was slightly stimulated with increasing water salinity, with an optimum at 100 mM NaCl. This was mainly due to enhanced tissue water content and succulence. Higher salinities considerably reduced plant growth, with maximum reduction of 82% observed at 500 mM NaCl. The plants were able to reduce the leaf water potential below the soil water potential. This was associated with substantial decrease in osmotic potential mainly by Na^+ and Cl^-. Interestingly, the plants were able to maintain favorable ion relations in their roots and juvenile leaves, where the metabolic demands are expected to be greatest, even under high NaCl salinity. The net photosynthesis rates were greatly decreased by high salinity, being 28% of initial control values at 500 mM NaCl. Salt-induced photosynthesis inhibition was accompanied with a decrease in transpiration rates but also with improved water use efficiency. Neither osmotic stress nor ion deficiency/toxicity appeared to be determinant for *C. quinoa* under high saline condition. Salt-induced growth reduction is presumably due to low photosynthate supply as a consequence of impaired photosynthetic capacity. Together, these indicate that *C. quinoa* is a promising salt-tolerant, in terms of biomass production, and can be grown productively under low to moderate saline condition up to 40% sws.

Keywords: *Chenopodium quinoa*; halophytes; ion relations; photosynthesis; salt-tolerance; osmotic adjustment.
Abbreviations: C_i, intercellular CO_2 concentration; D_r, dark respiration; E, transpiration; La, adult leaves; L_c, light compensation point; Lj, juvenile leaves; LMA, leaf mass to area ratio, L_s, saturation irradiation; N_P, Net photosynthetic rate; *PWUE*, photosynthetic water use efficiency; R, root; Sa, adult stems; $SA_{K:Na}$, Selective absorption; Sj, juvenile leaves; S_r, Stomatal resistance; sws, seawater salinity; TSC, Total soluble carbohydrates; Φ_c, apparent quantum yield of photosynthetic CO_2 assimilation

Introduction

Salinity is one of the most widespread environmental threats to global crop production, especially in arid and semi-arid climates, where land degradation, water shortage and population growth are already a major concern (Munns and Tester, 2008; Geissler et al., 2010). More than 800 million ha of land is salt-affected, which is over 6% of the world's land area (Rengasamy, 2006). Worldwide, salt-affected area is increasing as more and more land is ultimately claimed and irrigated for agricultural production to meet the exponential population growth (Lambers, 2003; Munns, 2005). Due to unsustainable irrigation practices, about 1.6 million ha/year of irrigated lands become saline and go out of production (secondary salinization) (Tanji, 2002). The global annual cost of salinity is likely to be well over US$12 billion (Qadir et al., 2008). Hence, the future of agricultural production will increasingly rely on our ability to grow plants on salt-affected and marginal lands using low (brackish or even saline) waters (Rozema and Flowers, 2008). Therefore, there has been a great incentive to increase salt tolerance of our conventional crops using genetic manipulation to allow greater yields in salt-affected areas (Flowers, 2004). However, this has been hampered by the multigenic nature of the trait and the seemingly scarce natural genetic variability in these plants (Läuchli and Grattan, 2007; Witcombe et al., 2008). Alternatively, exploration of naturally occurring high salt-tolerant plants "halophytes" for sustainable crop production are now being promoted, particularly for economic interests (food, fodder) or ecological reasons (soil desalinisation, dune fixation, CO_2-sequestration) (Boer and Gliddon, 1998; Lieth and Mochtschenko, 2002; Reddy et al., 2008). Utilization of halophytes as cash crops would help valorizing highly salinized zones and mediocre waters unsuitable for conventional agriculture (Rozema and Flowers, 2008). One promising species that has a high potential to become a cash crop is *Chenopodium quiona* Willd. (Chenopodeaceae). Quinoa has been an important food source in the Andean region for thousands of years (Jacobsen et al., 2009; Koyro and Eisa, 2008; Hariadi et al., 2011). Recently, it gains

worldwide attention because of its extraordinary tolerance to various environmental stress conditions like soil salinity, acidity, drought, frost, etc. (Maughan et al., 2009; Hariadi et al., 2011). Apart from this, its grains is a rich source of a wide range of minerals (Ca, P, Mg, Fe and Zn), vitamins (B_1, B_9, C and E), oil containing large amounts of linoleate, linolenate and natural antioxidants and high quality protein containing ample amounts of essential amino acids such as lysine and methionine (Koyro and Eisa, 2008; Abugoch et al., 2009). The nutrition value of quinoa seeds is reported to meet and surpass that recommended by the World Health Organization (WHO) (Hirose et al., 2010). Furthermore, its leaves are widely used as food for human and livestock (Weber, 1978; Aufhammer, 2000). Due to its high nutritional value, quinoa attracted the attention as alternative crop worldwide and has been chosen by the Food and Agriculture Organization as one of the crops destined for food security in this century (Mujica et al., 2001). Furthermore, quinoa is being considered a promising crop in NASA's Controlled Ecological Life Support System (CELSS) for long-duration manned spaceflights (Bhargava et al., 2005). In spite of overwhelming interest from agronomists towards quinoa, very little is known about its salt-tolerance level (threshold), growth response and seed yield under saline irrigation. Deeper understanding of individual physiological, biochemical and structural mechanisms that determine salt tolerance in *C. quinoa* is a prerequisite for its sustainable utilization as non-conventional crop using alternative water sources on marginal lands (Koyro, 2006). As well known, salt tolerance comprises an array of interconnected morphological, physiological and biochemical mechanisms on whole plant, tissue, and cellular/molecular levels (Ashraf and Harris, 2004; Tammam et al., 2008; Geissler et al., 2009a). These mechanisms are related to the four major constraints of salinity on plant growth, i.e., osmotic effects, restriction of CO_2 gas exchange, ion toxicity, and nutritional imbalance (Koyro, 2006; Geissler et al., 2009a). To withstand osmotic constraints, plants have to be more restrictive with water loss by a sensitive stomatal closure response. This, in turn, entails that gas exchange be kept low due to a restricted availability of CO_2 for the carboxylation reaction (stomatal limitation) (Huchzermeyer and Koyro, 2005; Flexas et al., 2007). In this field of tension, the fine-tuned control of H_2O/CO_2 gas exchange is crucial for plant growth and biomass production under this condition (Romero-Aranda et al., 2001; Gulzar et al., 2005). As gas exchange has not been studied in quinoa as far as we know, it is quite important to evaluate plant growth in connection with CO_2/H_2O gas exchange, especially at salinity tolerance threshold. Regarding the other two constraints mentioned above, high NaCl concentrations adversely affect the acquisition of essential nutrients as Na^+ competitively inhibits K^+ and Ca^{2+} uptake, whilst Cl^- restricts anions uptake (Tester and Davenport, 2003; Liu et al., 2006; Tammam et al., 2008), disturbing ion homeostasis within the plant. Moreover, salinity may create specific ion toxicity as disproportionate presence of Na^+ and Cl^- in cellular and intracellular compartments inhibits many enzymatic systems, altering a wide range of important metabolic processes that plant growth is crucially depending on (Blaha et al., 2000; Munns, 2005). Adaptation mechanisms should therefore contribute to re-establish the homeostatic conditions needed for inward net flux of water and ion uptake. One major aspect of plant adaptation to saline environments is the utilization of massive accumulation of inorganic ions (mainly Na^+ and Cl^-) to adjust osmotically (Ottow et al., 2005). Osmotic adjustment (OA) in terms of salt accumulation is energetically efficient, but requires a combination of several tolerance and/or avoidance strategies that act in concert to avert ion toxicity and imbalance (Munns 2005; Wang et al., 2007; Koyro et al., 2011). In consideration of this background, the present study aimed mainly at monitoring salt-induced responses of *C. quinoa* to get precise insights into the individual physiological mechanisms that may be involved in salt tolerance and to find out which of the above-mentioned four growth constraints restrict(s) salinity tolerance of *C. quinoa*.

Results

Growth and water relations

The overall growth was slightly (not significantly, $P \leq 0.05$) greater for plants grown at 20% sws than for plants at lower or higher salinities (Fig. 1a). Low salinity level (100 mM NaCl) led to 10% increase in the plant fresh weight relative to controls. Salt-induced growth stimulation was mainly due to enhanced shoot rather than root fresh weights (Fig. 1a). Salt tolerance threshold (the initial significant reduction in the maximum expected yield, Shannon and Grieve, 1999) was observed at 200 mM NaCl, while C_{50} (water salinity resulting in 50% growth reduction) was at 300 mM NaCl (Fig. 1a). High salinity treatment led to 82% reduction in plant fresh weight compared to relative controls. Although *C. quinoa* plants survived and completed their life cycle even at full strength salinity (500 mM NaCl), some visual symptoms of ion deficiency and/or toxicity were observed on the adult leaves. Leaf mass to area ratio (LMA) was distinctly increased with elevating water salinity, while this effect was much more pronounced for the adult leaves compared to the juvenile ones (Fig. 2a). Seed yield (g/plant) was significantly decreased as water salinity rose, with maximal reduction being 97% at the highest water salinity (Fig. 1b). Leaf water potential of *C. quinoa* was significantly ($P \leq 0.05$) decreased from –0.6 MPa under control conditions to about −5MPa at salinity of 500 mM NaCl (Fig. 3). Whatever the salinity treatment, leaf water potential was lower than that of the rooting medium (Fig. 3). Similarly, the osmotic potential of all plant organs was gradually declined as water salinity rose (Fig. 4). The reduction of the osmotic potential (osmotic adjustment) was accompanied with excessive accumulation of Na^+ and Cl^- (Fig. 4). In control plants, sodium and chloride accounted for only a small part (less than one third) of the osmotic potential in all plant organs. In contrast, these ions were the dominating solutes under saline conditions and accounted for about 45, 87 and 61% of OA in roots, stems and leaves respectively (Fig. 4).

Mineral ion contents

NaCl salinity induced significant increases in tissue Na^+ contents by 10 to 21 folds; while this effect was more pronounced in the leaves (Fig. 4). Similarly, tissue Cl^- concentration was increased as external salinity rose. Full-strength salinity treatment led to approximately 12 and 6 folds increases in the tissue Cl^- concentration of the leaves and roots respectively relative to controls (Fig. 4). Expressed on the leaf fresh weight, Na^+ excretion via bladder hairs was also enhanced from 0.06 and 0.2 $\mu m\ g^{-1}$ (controls) to 0.3 and 1.36 $\mu m\ g^{-1}$ (500 mM NaCl) for adult and juvenile leaves respectively (Fig. 5c). Untreated control plants exhibited high K^+ concentrations which accounted for about 44.8, 50.8 and 48% in the OA of roots, stems and leaves respectively. K^+ contents of all plant organs were dropped in response to elevating water salinity, with more severity in the juvenile

Table 1. Effect of elevated seawater salinity on the net photosynthesis rate (N_P), transpiration rate (E), photosynthetic water use efficiency ($PWUE$), Stomatal resistance (S_r) and ratio of internal to external CO_2 concentration (C_i/C_{an}) of *C. quinoa*. All of these values are at the light saturation point of photosynthesis.

NaCl Treatments	N_P [µmol m^{-2} s^{-1}]	E [mol m^{-2} s^{-1}]	$PWUE$ A/E	S_r [s cm^{-1}]	C_i/C_{an}
Control	59.39±10.79^{a}	16.05±4.51^{a}	3.81±0.68^{a}	0.63±0.21^{a}	0.60±0.05^{a}
100 mM	45.83±11.47^{b}	12.86±3.48^{b}	3.61±0.46^{b}	0.84±0.30^{b}	0.58±0.05^{b}
200 mM	31.72±7.23^{b}	10.19±2.51^{b}	3.16±0.42^{b}	1.44±0.54^{b}	0.51± 0.04^{b}
300 mM	24.72±6.68^{cd}	5.81±1.16^{cd}	4.32±1.49^{a}	2.01±0.44^{cb}	0.44±0.18^{ab}
400 mM	21.33±3.63^{cd}	5.07±0.98^{d}	4.27±0.56^{a}	2.75±0.53^{c}	0.38±0.04^{b}
500 mM	17.13±2.52^{c}	2.84±0.76^{c}	6.34±1.29^{b}	4.84±1.05^{c}	0.18±0.13^{b}

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by LSD test. Each mean represents nine replicates.

Table 2. Calculated photosynthetic efficiency (Φ_c), dark respiration (D_r), light compensation point (L_c) and light saturation point (L_s) under control and high NaCl salinity conditions.

NaCl Treatments	Φ_c [µmol CO_2 µmol^{-1} Quantum]	D_r [mol m^{-2} s^{-1}]	L_c [µmol m^{-2} s^{-1}]	L_s [µmol m^{-2} s^{-1}]
Control	0.1562	-4.57	28.40	1149.43
500 mM	0.0233	-5.09	197.43	2470.47

SD values and significances were not determined because Φ_c, D_r, L_c, and L_s were calculated once for each treatment with the help of an exponential function (Schulte et al., 2003), based on all 9 measurements at 0, 200, 500, 1000, 1500, and 2000 µmol photon m^{-2} s^{-1}, respectively.

leaves. High salinity level led to 40, 35 and 70% reductions in K^+ concentrations of the root, adult and juvenile leaves respectively (Fig. 4). As a consequence, the K^+/Na^+ ratio was sharply decreased from 15, 12 and 6 (controls) to 0.2, 0.08 and 0.05 (500 mM NaCl) for root, adult and juvenile leaves respectively (Fig. 5a).

Total soluble carbohydrate (TSC) contents

NaCl stress caused a significant increase in the total soluble carbohydrates (TSC) in all plant organs (Fig. 6). This effect was especially pronounced in the root tissues, where TSC content was more than 3 times higher at seawater salinity than in the control. Despite these increments in TSC, their contribution to the OA was declined from 6–19% (control) to 6–14% (high salinity) (Fig. 4).

Photosynthetic pigments and CO_2 gas exchange

In tendency, chlorophyll a & b contents of the full-expanded juvenile leaves (based on leaf area) were transiently reduced as external water salinity increased (Fig. 2b). This effect was most severe at 300 mM NaCl salinity, where significant reductions of about 28 and 40% in Chl(a) and Chl(b) respectively were observed. Further increase in water salinity slightly enhanced the pigment contents (Fig. 2b). High water salinity treatment led to 10 and 15% reductions in the Chl(a) and (b) respectively. Consequently, the chlorophyll a/b ratio showed a significant increase as water salinity rose up to moderate level. This ratio was not significantly affected by high salt treatment. Elevating water salinity significantly and steadily inhibited the net photosynthetic rate (N_P) at light saturation (A max), with maximum reduction being 72% at the highest salinity level (Table 1). In contrast, increasing water salinity strongly increased the stomatal resistance (S_r). At the highest salinity level, there was approximately an 8-fold increase in the stomatal resistance relative to control plants (Table 1). Structural investigations (Fig. 7) showed that most stomata on the adaxial and abaxial sides of the high NaCl-treated leaves were closed. Increasing water salinity led to a strong reduction in plant transpiration rate (E), which reached minimum level at the highest salinity treatment (Table 1). This led consequently to a significant increase in the photosynthetic water use efficiency ($PWUE$). Compared to the controls, $PWUE$ was increased by about 100% in plants grown at the highest salt concentration (Table 1). Salinity distinctly reduced the ratio of the internal to external CO_2 concentration (C_i/C_{an}) from 0.6±0.05 under control conditions to 0.18±0.13 at 500 mM NaCl treatment. The calculated photosynthetic efficiency (Φ_c) declined markedly as water salinity rose, being lowest (0.0233 µmol CO_2 µmol^{-1} Quantum) at the highest salinity treatment (Table 2). The

light saturation point (L_s) increased from 1149.43 µmol $m^{-2}s^{-1}$ under control conditions to 2470.47 µmol m^{-2} s^{-1} at full-strength water salinity (Table 2). Similarly, the light compensation point (L_c) increased gradually with increasing NaCl concentration in the nutrient solution, reached maximum at the highest water salinity (Table 2).

Discussion

The growth of *C. quinoa* was slightly stimulated with increasing water salinity, reaching a peak at 100 mM NaCl (Fig. 1a). This salinity level could be, therefore, considered as the optimal salinity. Salt-induced growth stimulation under moderate salinities has been reported previously for *C. quinoa* (Hariadi et al., 2011) and many other halophytic species (Debez et al., 2003; Ramos et al., 2004; Redondo-Gómez et al., 2006). Growth stimulation in response to moderate salinities might be largely the consequence of increased tissue water content (Prado et al., 2000; Khan et al., 2005). This is supported by the trends of water content and succulence in *C. quinoa*, which correlated with those of the plant fresh weight (data not shown). Salinity higher than 100 mM NaCl considerably reduced plant growth, with most adverse effects at seawater salinity (Fig. 1a). Nevertheless, this species was able to survive at about sea-level concentrations. Inhibition of new leaf initiation and the formation of small leaves, some with symptoms of nutrient disorders might contribute to the low fresh weight observed at this salinity level. While salinity tolerance threshold occurred at salinity level of 200 mM NaCl, the C_{50} was at 300 mM NaCl (Fig. 1a). Reduced biomass as a response to high substrate salinity is quite common in halophytes (Uchiyama, 1987; Ungar, 1996; Wang et al., 1997; Koyro et al., 2006; Geissler et al., 2009a). Together, these results indicate that *C. quinoa* is facultative halophyte, with high potentiality in terms of biomass production under moderate saline condition. The initial effect of salinity on plant growth is due to an osmotic stress, resulting from low substrate water potential (Munns, 2005). However, results of this study showed clearly that *C. quinoa* plants could efficiently lower their leaf water potential below the substratum water potential as a consequence of decreased tissue osmotic potential (Figs. 3 and 4). This implies that *C. quinoa* was able to effectively adjust osmotically, maintaining a positive water balance in response to water salinity. This can be inferred from the improvement of plant water status (increasing succulence) (Fig. 2a) and is in conformity with earlier observations on *C. quinoa* (Hariadi et al., 2011) and other halophytes (Debez et al., 2006; Koyro et al., 2008; Geissler et al., 2009a). Osmotic adjustment (OA) was associated with a substantial accumulation of Na^+ and Cl^- in all plant organs (Fig. 4), divulging that *C. quinoa* behaves –at the whole plant level- as a salt-includer, utilizes the controlled Na^+ and Cl^- uptake to adjust osmotically (Ueda et al., 2003; Ben Amor et al., 2005). OA by massive accumulation of inorganic ions has been amply reported in many halophytic species and considered to be less energy (carbon) demanding than OA by the *de novo* synthesis of organic solutes (Gulzar et al., 2005; Marcum, 2006; Koyro et al., 2011). However, salt accumulation in excess of what is required can lead to ion toxicity and/or imbalance. This strategy must be, therefore, accompanied by an efficient tolerance and/or avoidance mechanism(s) to regulate the internal Na^+ and Cl^- concentrations (Munns, 2005; Koyro et al., 2006). Yet, the ability of *C. quinoa* to thrive even at 500 mM NaCl implies that this species exerts some strict control over ion uptake, transport and compartmentation within the whole plant to prevent a

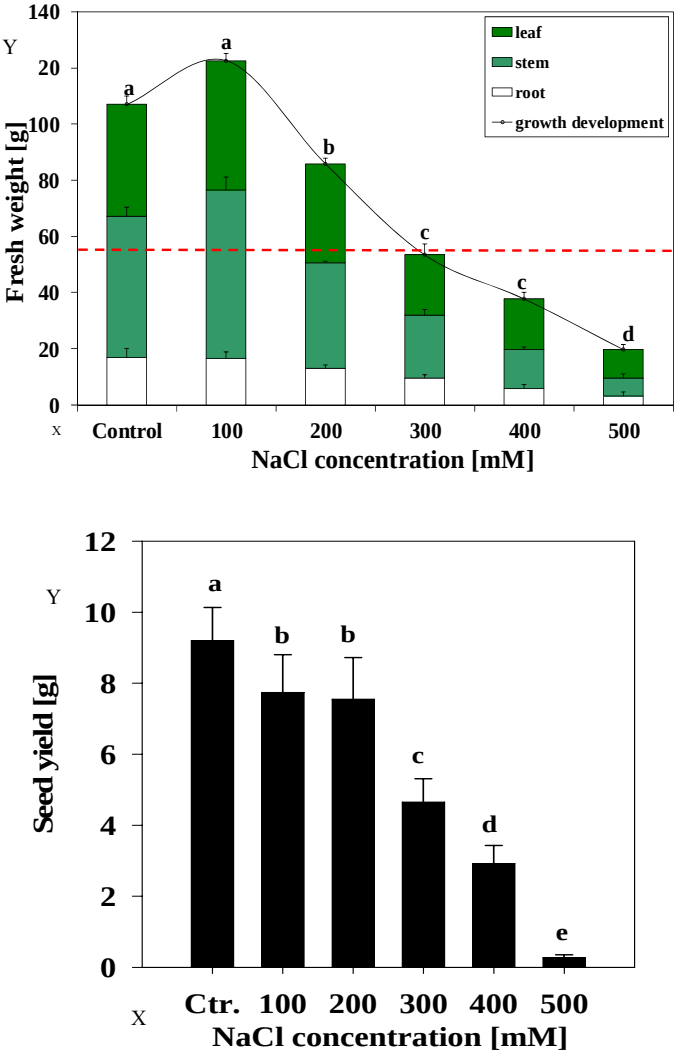

Fig 1. Effect of water salinity level on plant development and growth of different organs expressed as fresh weights (a) and seed yield (b). The dotted line in Figure (a) marks the C_{50} values. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P \leq 0.05$, LSD test.

continuous build-up of NaCl in the actively metabolic tissues (Munns and Tester, 2008). In accordance with previous investigations, Na^+ was shown to be preferentially accumulated in the shoot parts (Fig. 4); thereby the plants avoid excessive ion accumulation in the root tissues (Koyro, 2000; Ashraf et al., 2006). This further confirms that *C. quinoa* adopts an inclusion strategy to tolerate high water salinity. Ion accumulation in the shoot parts is possibly attributable to an enhanced selective ion uptake in favor of K^+ over Na^+ ($SA_{K: Na}$) at the root level on the one hand (Fig. 5b) and a high transport capacity in favor of Na^+ versus K^+ from the root to the shoot on the other hand. It is worth noting that the juvenile leaves exhibited comparatively lower Na^+ concentrations as the adult ones, suggesting that the adult leaves might function as ion sinks to prevent salt accumulation in the actively growing and photosynthesizing juvenile leaves (Koyro, 2000; Liu et al., 2006). This

assumption can be inferred from the fact that old leaves exhibited signs of sodium and/or chloride toxicity before finally dying off. Another strategy for counteracting ion specific effects in *C. quinoa* plants is their ability to remove or compartmentalize salt surplus, mainly Na^+ and Cl^-, from the leaf blades into the bladder hairs located on the leaf surfaces, where it can be washed off by rain. Here, Na^+ and Cl^- excretion via the bladder hairs were increased incrementally as substrate salinity rose (Fig. 5c). This result concurs with previous observations for many *Atriplex* species (Schirmer and Breckle, 1982; Waisel, 1991). Comparing salt contents of bladder hairs and leaf tissues at the highest salinity level reveals that about 0.05 and 0.7% of the total leaf Na^+ contents were excreted into the bladder hairs of the adult and juvenile leaves respectively. These observations indicate the role that the bladder hairs could play in removing salt from salt sensitive metabolic sites of the leaves, particularly the juvenile ones (Tester and Davenport, 2003; Naidoo et al., 2008). High Na^+ excretion from juvenile leaves has been attributed to a higher density of bladder hairs on the juvenile leaves as compared to the adult ones, since the initiation of these bladders start only in the early stages of the leaf development (Kelley et al., 1982). This insinuates that the basic role of these bladders is to protect the young developing leaves. Low density of bladder hairs on the adult leaves proves that in these organs ion dilution as a result of increasing leaf succulence is the main strategy to avoid the toxic effects of harmful ions (Mimura et al., 2003; Debez et al., 2006; Geissler et al., 2009a). Sodium accumulation was associated with a sharp decrease in K^+ concentrations in all plant tissues, with less severity on the root (Fig. 4). Similar results have been observed previously, and interpreted as a result of competition between K^+ and Na^+ at the level of absorption sites (Hasegawa et al., 2000; Zhu, 2003) or due to the changes in the membrane integrity caused by the displacement of Ca^{2+} by Na^+ (Cramer et al., 1985; Tester and Davenport, 2003). However, salt-induced reduction in K^+ contents does not necessarily mean that there was a potassium deficiency, because in many dicots (Shennan et al., 1987; Venema et al., 2003) the osmotic function of potassium, magnesium or calcium in the vacuole can be substituted by sodium without any growth depression, so that the substances mentioned above can be increasingly used for specific functions in the cytoplasm. Salinity tolerance has been reported to be related with the ability of the plant to maintain an appropriate K^+/Na^+ ratio rather than simply maintaining low Na^+ concentrations (Silveira et al., 2001; Shabala and Cuin, 2008; Gorai et al., 2010; Bayat et al., 2011). Here, K^+/Na^+ ratio was significantly lowered with elevating water salinity as previously observed in many chenopodiaceous species (Jeschke and Stelter, 1983; Ramos et al., 2004). Interestingly, *C. quinoa* was able to maintain favorable K^+/Na^+ relations in their root and juvenile leaves at least in part under moderate salinities (Fig. 5a). This appears advantageous insofar as it provides a way of directing much of the available K^+ to the most actively growing sites, where the metabolic demands are expected to be greatest and Na^+ sensitivity is highest. The ability of this species to maintain a substantial growth at high salinity, though, with some signs of nutritional imbalance (particularly on the adult leaves) may indicate that there is no impaired ion homeostasis in the cytosol. One can assume that most of harmful ions are compartmentalized into the vacuoles, thereby preserving intracellular ion homeostasis necessary for cytoplasmic metabolic activity and increase cellular osmolality to counter osmotic stress (Wang et al., 2007). In order to verify this

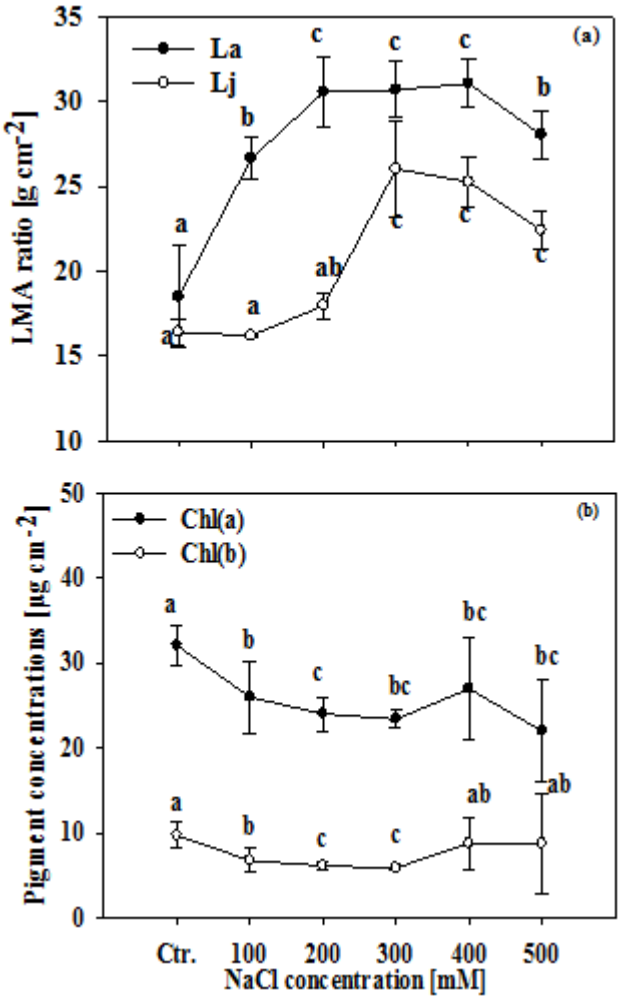

Fig 2. Effect of increasing NaCl salinity on leaf mass to area ration (a) and Chlorophyll concentrations (b) of adult and juvenile leaves. Each value represents the mean of nine replicates and the bars represent standard errors. Bars with the same letter are not significantly different at $P \leq 0.05$, LSD test.

thesis, it would be important to measure the mineral content of different cell compartments (e.g. by EDX analysis). As mentioned above, ion sequestration is a cost-effective mechanism in respect of the amount of energy and resources spent. However, this mechanism by itself is an energy-consuming process and usually accompanied by organic solutes synthesis (extra energy requirements) in the cytosol to counteract the increased osmolality of the vacuole (Huchzermeyer and Koyro, 2005; Ashraf et al., 2006). An important group of compatible solutes studied in *C. quinoa* is the carbohydrates. The concentration of total soluble carbohydrate (TSC) was significantly increased with elevating water salinity in all plant organs, especially in the roots (Fig. 6). Carbohydrate accumulation has been frequently reported as a response to salinity stress and thought to play an important role in the osmotic adjustment in many salt-tolerant plants (Popp and Smirnoff, 1995; Bajji et al., 1998; Murakeozy et al., 2003). However the adaptive value of TSC accumulation in salt tolerance of *C. quinoa* is questionable, since its involvement in the OA was either decreased from 19 to 14% (root) or did not change (shoot) in

response to high water salinity (Fig. 4). But this does not preclude its important role in the osmoregulation, because it is mainly synthesized and restricted in the cytosol, providing merely about 10% of cell volume. Thus the importance of TSC as cytoplasmic osmolytes might be higher than is suggested here from contents on the basis of total dry weight. Apart from their osmotic function in the cytoplasm (Adams et al., 2005; Lee et al., 2008), they might serve as molecular chaperones (Hasegawa et al., 2000; Liu et al., 2006). TSC accumulation could hardly be explained by a disturbance in carbohydrate metabolism regulated by various synthesizing and degrading enzymes that may be ion-specifically controlled (Singh et al., 1996; Toroser and Huber, 1997). Accumulation of TSC doesn't, however, exclude the possibility that high salinity might reduce the net photosynthetic capacity (James et al., 2002; Netondo et al., 2004). As shown in Table 1a, the net photosynthesis rate (N_P) was greatly decreased. The same trend was earlier reported in many halophytes (Bayuelo-Jimenez et al., 2003; Geissler et al., 2009b). Because plant productivity and yield are inextricably related to the photosynthesis capacity, this inevitably led to a significant reduction in the seed yield by about 97% at high water salinity level (Fig. 1b). In previous studies on rice and wheat plants, reduction in seed set (yield) under saline conditions was attributed to a reduction in photosynthesis and lower activity of starch synthase towards grain filling and seed set, resulting in lower grain yield (Sultana et al., 1999; Khan and Abdullah, 2003). Similar results were published for the halophyte *Plantago crassifolia* (Boscaiu et al., 2005). Salt-induced photosynthesis inhibition was coincided with a strong decrease in the transpiration rate (E) (Table 1), contributing to a positive water balance. Similar characteristics of water conservation have also been reported for many halophytic species under saline conditions (Carrol et al., 2001; Liu and Stützel, 2002; Debez et al., 2006). Such a low transpiration rate may reduce salt loading into the leaves, particularly the juvenile ones, and hence prolong the leaf lifespan by maintaining salts at subtoxic levels (Everard et al., 1994; Koyro, 2006). An aspect that deserves further comment is that salt-induced reduction in the transpiration rate was proportionally larger than those in the photosynthetic rates, leading to improved photosynthetic water use efficiency (*PWUE*) (Table 1). This would be an advantage, bestowing long-term survival of plants under stress conditions (Naidoo and Mundree, 1993). Enhanced *PWUE* in response to elevating water salinity has already been reported for many halophytic species (Downton et al., 1985; Ayala and O'Leary, 1995). It is likely that salinity impacted the photosynthesis of *C. quinoa a priori* by an enhanced stomatal closure which leads to a substantial reduction of CO_2 diffusion to the carboxylation sites. This assertion is supported by the linear proportionality of the net photosynthesis (N_P), the transpiration rate (E), the stomatal resistance (S_r) and the internal CO_2 concentration (Table 1). Similarly, a positive correlation between photosynthesis and stomatal conductance has been observed in many salt-tolerant species (Wang et al., 1997; Qiu et al., 2003). However, severe reduction of photosynthesis at high salinity levels may attribute to the biochemical and photochemical capacities of the leaf (non stomatal limitations) (Netondo et al., 2004; Sobrado, 2005). This may be due to an inhibition (synthesis or activity) of several enzymes related to the photosynthesis such as Rubisco, which has been reported to decrease under salt stress (Rivelli et al., 2002). Light intensity required to saturate the photosynthesis (L_s) in *C. quinoa* was significantly increased with increasing water salinity (Table 2). This is

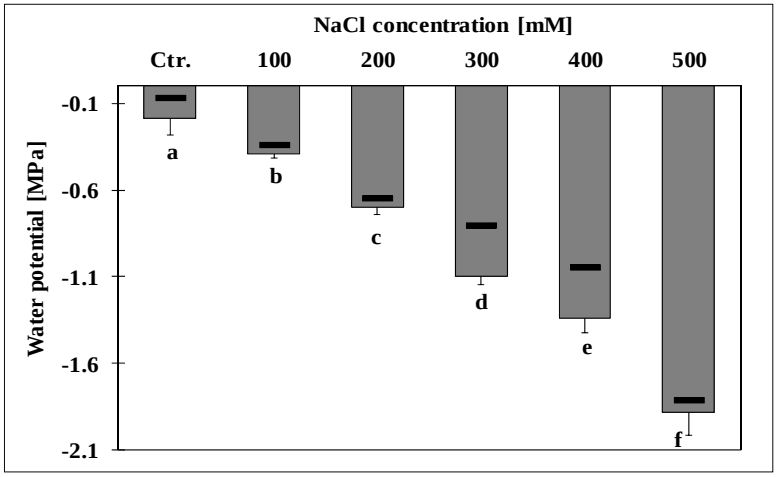

Fig 3. Leaf water potential of *C. quinoa* at varying water salinities. Black dashes indicate substrate water potential. Each column represents the mean of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P \leq 0.05$, LSD test.

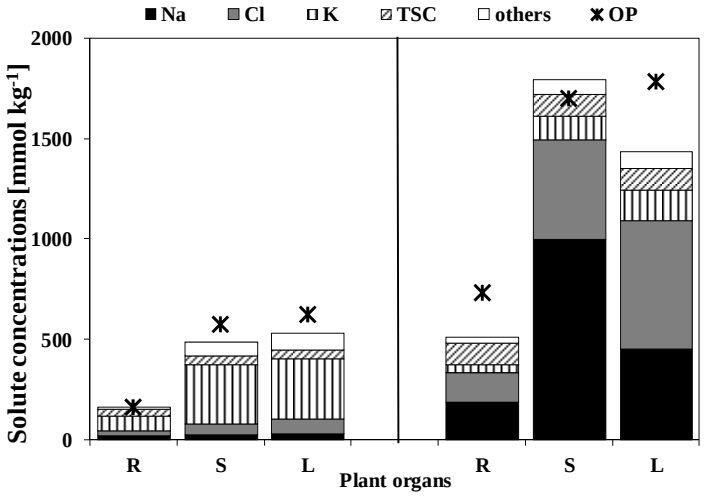

Fig 4. Concentrations of osmotically active substances in different plant organs at control (a) and 500 mM NaCl (b). R, roots; S, stems; L, leaves; Na, sodium; Cl, chloride; K, potasium; TSC, total soluble carbohydrates; and other (Ca, Mg, SO_4, PO_4, oxalate, amino acids). * Symbols mark the corresponding osmotic potential of the press sap.

likely to be a consequence of decreased chlorophyll (a & b) concentrations per unit leaf area (Fig. 2b). NaCl-induced decrease in chlorophyll content has been widely reported (Koyro, 2006; Geissler et al., 2009b). The depressive effects of salinity on leaf chlorophyll contents might be attributed to either a diminished chlorophyll biosynthesis (due to nutrient deficiency) or enhanced chlorophyllase activity (Ashraf and Bhatti, 2000). Results of this study showed that the reduction in Chl(b) was proportionally higher than that of Chl(a) and subsequently the ratio Chl a/b was slightly increased with increasing salinity. Reduced Chl(b) content (mostly located in the LHC) could be interpreted as an adaptation in the LHC capacity, which in turn resulted in a reduction (optimization) of the photosynthetic efficiency (Φ_c) (Table 2) and hence of oxidative stress (Moorthy and Kathiresan, 1999; koyro, 2006). Salt-induced reduction in the total chlorophyll

contents is considered to be an adaptive mechanism to cope with salt stress, since it may lead to decrease the over reduction of the photosynthetic electron transport and hence the generation of reactive oxygen species (ROS) (Wang et al., 2003; Christian, 2005). Increasing leaf succulence combined with the reduction in chlorophyll content observed in quinoa leaves under seawater irrigation may lead to a reduction of the flow of electrons through the photosystem (reduction of apparent quantum efficiency) (Table 2). Commensurate with the reduction in N_P, the light compensation point (L_c) was also increased with elevated water salinity (Table 2). This might be due to either a large stimulation of respiration or some breakdowns in the carbon mechanism. Similar results were found by Lutts et al. (1996) and El-Shintinawy (2000). Low net photosynthesis (N_P) might be related to enhanced respiratory costs (Table 2) required for salt economy (ion compartemntation and excretion), biosynthesis of compatible solutes and repair of cell structures under saline conditions. This would presumably occur at the expense of net CO_2 fixation, resulting in a reduced overall growth (Schwarz and Gale, 1981). However, salt-induced growth reduction is assumed to be an adaptive mechanism for the survival under saline condition because it allows the plant to rely on multiple resources to cope with salinity stress (Zhu, 2001).

Material and methods

Plant materials and growth conditions

Seeds of *C. quinoa* willd cv. *Hualhuas* (origin: The International Potato Center, CIP, Lima, Peru) were washed several minutes with running tap water and imbibed for one day in the dark (25°C) in an aerated 0.2 mM $CaSO_4$ solution. The seeds were then transferred onto a Petri dish with wet filter paper (0.2 mM $CaSo_4$) for germination in a dark growth cabinet at 25°C. Seven days after germination, the plants were potted into soil (type LD 80, Fa Archut, Vechta, Germany). After further 2 weeks the young seedlings were transplanted into a hydroponics quick-check system (Koyro, 2003). The plants were irrigated with a basic nutrient solution (Epstein, 1972), and the pots were permanently aerated. Plants were grown under photoperiodic conditions (16h light/8h dark) in an environment controlled greenhouse (Giessen, Germany). Temperatures were 25±2°C during the day and 15±2°C during the night. Relative humidity ranged from 45 to 70%. Irradiation intensity was in the range of 100 µ Em^{-2} S^{-1} at the plant level.

Salt water treatments

To avoid salt shock injuries, stepwise application of NaCl to the basic nutrient solution began after a period of one week by raising the salinity of the solution in steps of 100 mM NaCl each day. There were altogether six treatments (10 replicates each): Control, 100, 200, 300, 400 and 500 mM NaCl (equivalent to 0, 20, 40, 60, 80 and 100% seawater salinity). Nutrient solutions were changed every 2 weeks to avoid nutrient depletion.

Samples and harvest procedure

Four weeks after salt application (6 weeks after transplanting into soilless culture), three plants from each treatment were randomly chosen to measure water potential and CO_2 gas-exchange. One week later, the same plants were harvested and immediately separated into roots (R), adult leaves (La), juvenile leaves (Lj), adult stems (Sa) and juvenile stems (Sj). The fresh weights of all plant organs as well as the leaf mass to area ratio (LMA) were directly captured. Representative specimens of about 500 mg from each plant organ were immediately ground in liquid nitrogen and then stored at -80°C for the quantitative chemical analysis. Seed harvesting

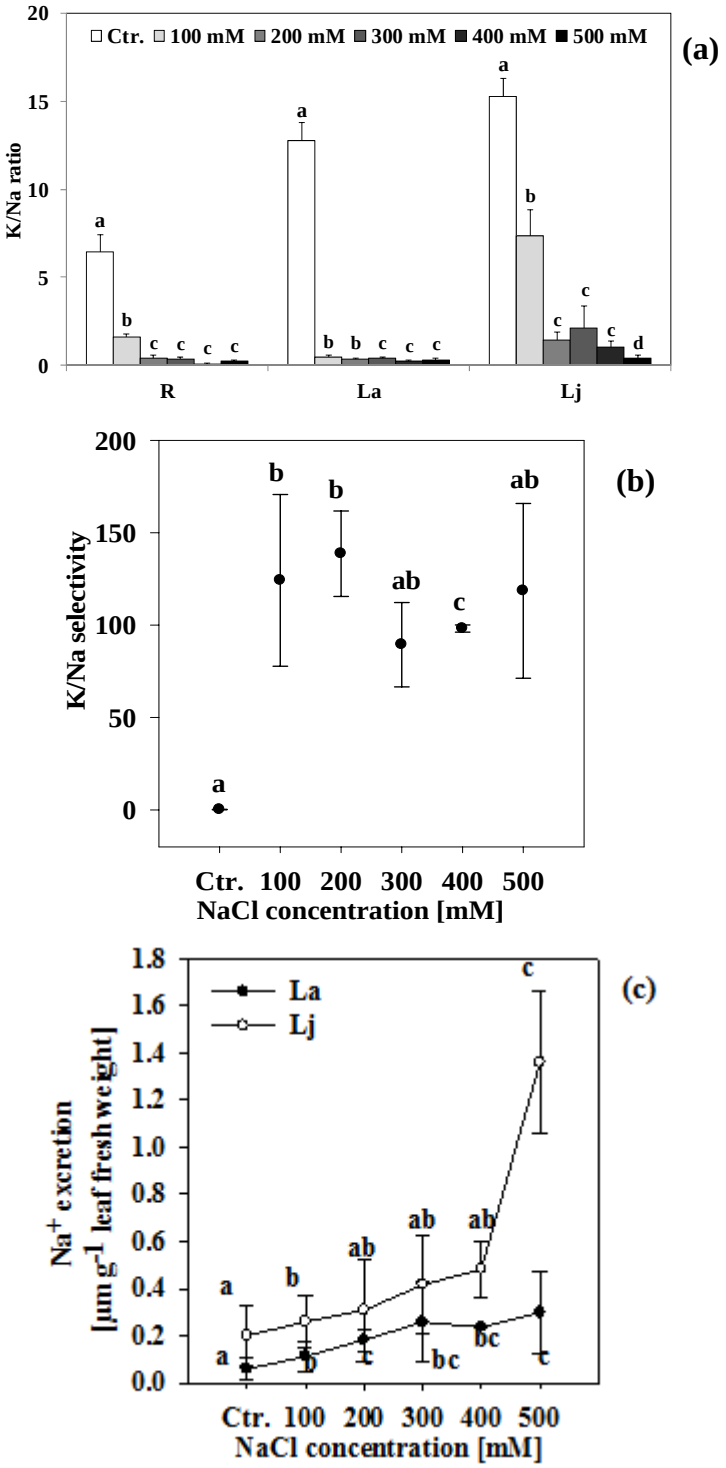

Fig 5. Effect of water salinity on K/Na ratio in different plant organs (a), selective ion uptake of K over Na (b) and Na excretion via bladder hairs of adult and juvenile leaves (c). Each value represents the mean of nine replicates and the bars represent standard errors. Bars with the same letter are not significantly different at $P \leq 0.05$, LSD test.

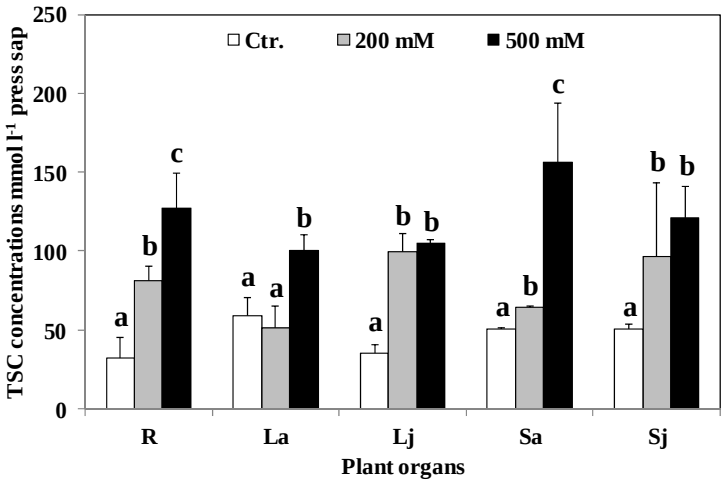

Fig 6. Effect of various NaCl salinities on total soluble carbohydrate contents of the roots (R), adult leaves (La), juvenile leaves (Lj), adult stems (Sa) and juvenile stems (Sj). Each value represents the mean of nine replicates and the bars represent standard errors. Bars with the same letter are not significantly different at $P \leq 0.05$, LSD test.

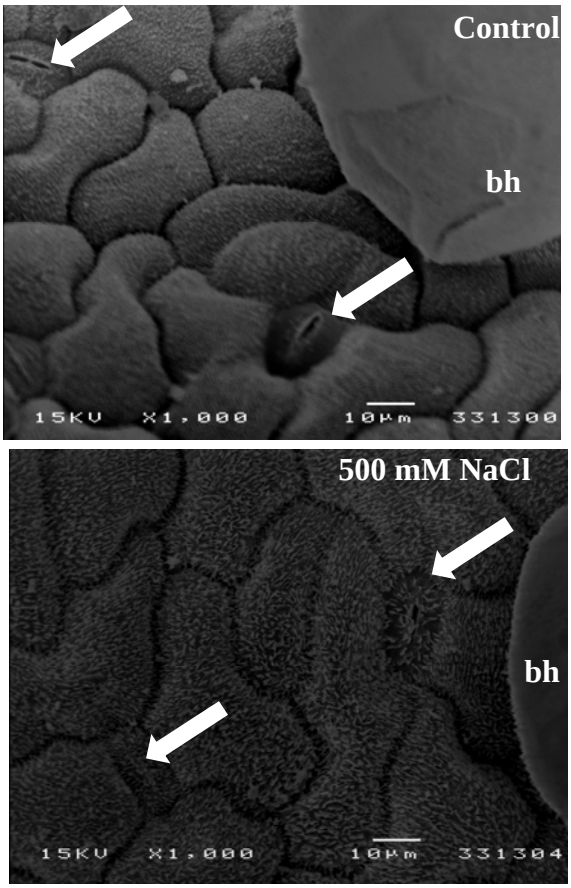

Fig 7. Representative scanning electron micrographs of the juvenile leaf surface at control conditions (left) and at 500 mM NaCl (right). Bh, bladder hairs. Note open stomata in untreated leaves and closed stomata in salt treated leaves (arrows).

took place 16 weeks after sowing date (11 weeks after salt application).

Determination of water and osmotic potentials

Leaf water potential was psychometrically measured on the abaxial surface of the intact leaves with a Dew point microvolt meter (Wescor Type HR 330, 218 WESCOR Inc, USA). The osmotic potential of the press sap of all plant organs was measured with the freeze-point depression method using a cryo-osmometer (Osmomat 030, Genotec GMBH, Berlin).

Photosynthetic pigments and CO_2 gas exchange

Chlorophyll a and b concentrations were spectrophotometrically determined 5 weeks after salt application in both adult and juvenile leaves according to Lichtenthaler and Wellburn, (1983). Gas exchange of the second or third uppermost fully expanded leaves was measured using a closed photosynthesis measurement system Li-COR 6200 (Li-COR, Lincoln, NE, USA) under different water salinity levels. All measurements were taken at atmospheric CO_2 concentration of 419.27±29.82 ppm, 30.84±0.67°C air temperature, and 35.7±2.87% relative humidity. A steady state light response curve was determined at photosynthetic photon flux density of 0, 500, 1000, 1500 and 2000 µmol m^{-2} s^{-1}) using a halogen light source (50 w). The light compensation point (L_c), dark respiration (D_r), saturation irradiation (L_s) and apparent quantum yield of photosynthetic CO_2 assimilation (Φ_c) were calculated using the exponential function reported by Schulte et al. (2003). Net photosynthetic rate (N_P), transpiration (E), stomatal resistance (S_r) and intercellular CO_2 concentration (C_i) were determined at saturating irradiation (1500 µmol photon m^{-2} s^{-1}, formulas for calculation in Li-Cor Inc., 1990), suggested being the light saturation point for photosynthesis.

Mineral contents

To determine ion contents of the bladder hairs, the adaxial and abaxial surfaces of the adult and juvenile leaves were rinsed in 25 ml distilled water (bladder hair fractions). About 500 mg of washed plant materials were dried for 48 h at 105°C and weighed, then ashed in a muffle furnace over night at 550°C and finally extracted with HNO_3 (32%). Ion contents in these extractions and in the bladder hair fractions were measured separately using atomic absorption spectrophotometer (Perkin Elmer model PE 2100). Selective absorption ($SA_{K:Na}$) for K^+ over Na^+ was calculated according to the following equation:
$SA_{K:Na}$= (available Na^+/K^+ in the soil)/(Na^+/K^+ in the root) (Pitman, 1965).

Total soluble carbohydrate (TSC) contents

Total soluble carbohydrates of all plant organs were photometrically assayed according to Kleber et al. (1987) and Volk (1996).

Statistical analysis

All data sets were subjected to a one-way-ANOVA analysis using SPSS for Windows statistical data analysis package (SPSS Inc., 2002, release 16, Chicago, IL, USA). Tukey´s post-hoc test was employed to determine if significant ($P \leq$

0.05) differences occurred between individual salinity treatments.

Conclusion

In conclusion the present findings and reasoning allow for the speculation that *C. quinoa* is a highly salt tolerant species in terms of biomass production, as it survives even at 100% sws. Salt tolerance in this species is largely conferred by a delicate balance among osmotic adjustment and ion accumulation. Neither osmotic stress nor ion deficiency/ toxicity seems to be determinant for *C. quinoa* under high saline condition. Growth reduction associated with a long-term high NaCl salinity is, presumably due to a low supply of photosynthate in the plant as a whole as a consequence of impaired photosynthetic capacity. This worse effect is aggravated by the diversion of photoassimilates away from the synthetic processes involved in cell growth to the synthesis of solutes for osmotic regulation, resulting into lower maximum grain yield. Finally, *C. quinoa* doesn't only offer the possibility of being an alternative promising cash crop under moderate salinities, but also, through an understanding of its physiology, may provide possible routes to enhance salt tolerance in other crops.

Acknowledgments

The authors wish to thank Prof. Dr. Christoph Müller, Institute of Plant Ecology, Justus-Liebig-Universität of Giessen, Germany for critically reading the manuscript. The authors are indebted to Mr. Wolfgang Stein, Mrs Angelika Bölke, and Mr Gerhard Mayer for technical Assistance.

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