Environmental and Experimental Botany 177 (2020) 104146

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Differential responses to salt stress in ion dynamics, growth and seed yield of European quinoa varieties

Viviana Jaramillo Roman[a,b], Leonardus A. den Toom[a], Carlos Castro Gamiz[a], Niels van der Pijl[a], Richard G.F. Visser[a], Eibertus N. van Loo[a], C. Gerard van der Linden[a,*]

[a] Plant Breeding, Wageningen University and Research, Wageningen, the Netherlands
[b] Graduate School Experimental Plant Sciences, Wageningen University, Wageningen, the Netherlands

ARTICLE INFO

Keywords:
Chenopodium quinoa
Salt stress tolerance
Na^+ exclusion
RGR
Survival and growth

ABSTRACT

Quinoa is a nutritious seed crop with a great potential to grow in saline soils. Here, we studied ion concentrations in quinoa tissues throughout the life cycle of the plant, and linked ion dynamics to responses in growth parameters, seed yield and efficiency of photosynthesis under salinity (0–400 mM NaCl). Ion dynamics changed from high ion exclusion (> 99 %, root contents lower than root medium and low accumulation of ions in the leaves) before flowering, to a build-up of ions during seed filling. This indicates a change in strategy in maintaining the necessary gradient of water potential from the root medium to the leaves. K^+ concentrations in leaves also increased by more than 100 % in response to prolonged severe salt stress, which may point to a role of this ion in leaf osmotic adjustment. Accumulation of ions in epidermal bladder cells did not contribute substantially to Na^+-exclusion as it was less than 6 % of the total Na^+ taken up in leaves. Growth under salt stress was mostly impaired by anatomical adaptations (reduced SLA), while initial light use efficiency (Fv/Fm) and NAR were not affected. The variety Pasto showed a "survival strategy" to high salinity with higher ion exclusion and a higher reduction in transpiration than the other varieties, at the expense of lower biomass and seed yield.

1. Introduction

Soil salinity is a major abiotic stress that seriously threatens plant growth and food security (Roy et al., 2011). In the coming decades, salt-affected agricultural areas will expand as a consequence of both climate change and poor land management (Daliakopoulos et al., 2016). Remediation of salt-affected land is necessary but will take years before standard food crops can be grown again, so the development of resilient crops that can survive and be productive on these conditions should complement remediation of saline soils.

Halophytes are plant species that are naturally well adapted to high salinity, and can survive, grow and reproduce under extreme saline conditions (Flowers and Colmer, 2008). However, most halophytes are of little interest for agriculture as their yields are too low or their biomass unsuitable as food or feed (Shabala, 2013). One of a few exceptions is quinoa (*Chenopodium quinoa*). Quinoa is considered one of the most salt tolerant crop species, even more tolerant than barley or wheat (Gonzalez et al., 2015). Originating from the Andean Altiplano, quinoa is adapted to a broad range of ecosystems and abiotic stresses including saline soils, drought and frost (Zurita-Silva et al., 2014). Quinoa grows optimally under low or no salinity, but it can still produce seeds at soil salt levels that equal or even surpass those of sea water, and is therefore classified as a facultative halophyte (Mishra and Tanna, 2017). The ability to produce relatively high yields on saline soils where other crops are highly affected or failing justifies the designation of quinoa as an essential crop to ensure food security (Zurita-Silva et al., 2014).

The highest reported soil electrical conductivity (EC) level at which quinoa was able to survive was 51.5 dS/m, while 50 % reduction in yield was found at an EC of 25 dS/m (Razzaghi et al., 2015). Some studies claim that optimal growth and performance of quinoa can be achieved between 10 and 20 dS/m (Adolf et al., 2013; Hariadi et al., 2011; Jacobsen et al., 2003), while others state that quinoa plants start to be affected at salinity levels of 8 − 10 dS/m (Geissler et al., 2015; Hirich et al., 2014). These differences point to the existence of a rich pool of genetic resources that can be used for breeding quinoa varieties with improved yield under high salinity. In addition, the remarkable resilience of quinoa may also provide new insights into salt tolerance mechanisms that can be extended as breeding targets for other species.

Salt tolerance is a complex trait that requires a coordinated response of the plant to withstand the osmotic and ionic stress that salinity

* Corresponding author at: PO Box 16, 6700AA, Wageningen, the Netherlands.
E-mail address: gerard.vanderlinden@wur.nl (C.G. van der Linden).

https://doi.org/10.1016/j.envexpbot.2020.104146
Received 26 February 2020; Received in revised form 25 May 2020; Accepted 5 June 2020
Available online 09 June 2020

imposes on the plant. Plant species have a variety of responses to overcome both. Salinity decreases the osmotic potential of the soil, which leads to decreased turgor pressure in root cells and consequently water loss (Julkowska and Testerink, 2015). To avoid water loss, a first response of the plant is to close stomata and reduce transpiration at the cost of lower cell extension rate and growth. The maintenance of turgor is also facilitated by decreasing the osmotic potential in the roots, which is achieved by increasing the concentration of osmolytes in tissues. Osmotic adjustment is an essential plant response to salt stress, and can be achieved by the synthesis of organic compounds, or the accumulation of Na^+ and Cl^- in a cost-effective manner (Munns et al., 2016). In addition to the challenge of transporting water under salt stress, the plant has to deal with the salt ions (Na^+ and Cl^-) that are taken up and that are toxic at high concentrations. Several strategies have been described with the main goal of keeping ion concentrations low in the cytosol, particularly in the mesophyll cells in the leaves. Ions can be excluded, or secreted, from root tissues back to the root medium, or retrieved from xylem parenchyma cells by specific and well-studied ion transporters, (like SOS1 and HKT type 1) (Møller and Tester, 2007). A recent review examines the implications of keeping ion concentrations in shoots of plants low (Munns et al., 2020). Maintaining low levels of ions in the shoot over a longer period of time requires a high level of Na^+ exclusion in plants (and Cl^- exclusion to a lesser extent). The longer a plant is exposed to high salinity, the more challenging it will be to maintain low shoot ion levels. However, only a few studies have examined dynamics of ion accumulation throughout plant development (Ashraf and Khanum, 1997; Sairam et al., 2002), and whether the high level of exclusion is sustained through the life cycle of plants remains unanswered.

This paper examines the dynamics of ion homeostasis from young plants until seed maturation in different tissues of quinoa plants. Several reports have studied physiological traits that might explain the high salt tolerance in quinoa and showed broad genetic diversity in the extent of exclusion of ions (mainly Na^+) from shoots (Hinojosa et al., 2018), but most of these studies focused on rather young plants and relatively short duration of salt stress (Adolf et al., 2012; Hariadi et al., 2011; Shabala et al., 2013; Ruiz-Carrasco et al., 2011). We evaluated a set of commercial varieties at several degrees of salinity severity throughout the crop cycle to identify potential strategies of quinoa to adapt to prolonged exposure to salt stress. The variety of responses of quinoa cultivars described here demonstrate that quinoa qualifies as a model crop for studying halophytic salinity tolerance mechanisms.

2. Materials and methods

2.1. Plant materials

Four European non-bitter (sweet) quinoa varieties were used in the different experiments described below: Atlas, Jessie, Pasto, a line (selRiobamba) selected from Riobamba (Riobamba has still some residual heterozygosity) and one dark-seeded, bitter variety (Red Carina). The varieties were bred at Plant Breeding, Wageningen Research (The Netherlands) and AbbottAgra (France) and are adapted to the Western European climate and photoperiod.

2.2. Experimental conditions and treatments

Three experiments were carried out in three consecutive years (2015–2017). All the experiments were performed using spaced plants in 3 L pots. The plants were irrigated with half-concentrated Hoagland's nutrients solution. Salt treatments started five weeks after sowing, when plants had four fully developed pairs of leaves. Salt was applied by incremental increases of 75 mM per day until the desired salt concentration was reached. Salt concentrations were monitored regularly by measuring the electrical conductivity in the leakage from the pots with a conductivity meter (Profline Cond 315i, Xylem Analytics, Germany). All the experiments were conducted at the Unifarm greenhouse facilities of Wageningen University & Research, The Netherlands between April and September under natural photoperiodic (long day) conditions. The greenhouse air humidity was set to a minimum of 80 %. When the incoming shortwave radiation was below 200 Wm^{-2}, additional lighting was supplied (100 Wm^{-2}). Light irradiance, air temperature, water content and electrical conductivity (EC) in the pots were monitored via wireless sensors (Flower Power™).

The first experiment (2015) aimed to evaluate the general performance of European sweet quinoa growing at different levels of soil salinity. The varieties Atlas, Jessie, Pasto and selRiobamba were grown at four different levels of salinity: 0, 100, 200 and 300 mM NaCl. The experiment was done in a screenhouse using vermiculite as substrate, and the pots were drained with saline solution frequently to maintain a stable level of salinity in the pots. Ten plants per variety were used for each treatment. Half of the replicates were harvested ten weeks after sowing, during the vegetative phase growth of the plants. The other half was harvested at seed maturity (20 weeks after sowing).

The second experiment (2016) included the same varieties used in Experiment 1, plus the dark bitter variety Red Carina, grown at high salinity levels: 300 and 400 mM NaCl. Eight plants per variety were used for each treatment. Half of the replicates were harvested at the onset of flowering (11 weeks after sowing). The other half was harvested at seed maturity (20–24 weeks after sowing). The experiment was done in the greenhouse using fine vermiculite (size 1) as substrate.

The third experiment (2017) was a time series experiment using the most contrasting varieties in terms of agronomical and salt tolerance related traits from Experiments 1 and 2 (Jessie, Pasto and selRiobamba) and a severe salt stress of 400 mM NaCl. Three replicates per variety were harvested at four different time points during the growing season: 9, 12, 16 and 20 weeks after sowing. As we encountered draining problems with fine vermiculite as substrate resulting in salt accumulation in the pots in the second experiment, we switched to course vermiculite (size 3) in this experiment.

2.3. Assessment of growth traits

Plant height was measured weekly. Plant developmental stages were scored weekly according to a cardinal scale adapted from Masterbroek et al. (2002) (Table 1). During each destructive harvest, the biomass of the plants was separated into above-ground biomass (stems, leaves, heads) and roots. Leaves were removed from the plant and separated into young leaves (one-third upper part of the plant) and old leaves (two-third lower part of the plant). Fresh weights of leaves, stems, heads and roots were recorded, and leaf area was measured using a leaf area meter (Li-3000 Area Meter, Li-Cor, Lincoln, NE, USA). Dry weights were determined after drying leaves, roots and stems in a forced-air oven at 70 °C, (seeds at 35 °C), until samples reached stable weights. During the vegetative growth of the plants (from the transplanting date: three weeks after sowing, until the first destructive harvest: nine weeks after sowing) relative growth rate (RGR, d^{-1}) and its specific components were calculated based on the linear relation

Table 1
Plant development stages in quinoa. Adapted from (Mastebroek et al., 2002).

Stage	Description
F1	Flower buds just visible
F2	Flower buds 1.0 cm
F3	First glomeruli show anthers
F4	50% glomeruli show anthers
F5	Wilted anthers
F6	Seeds watery ripe/ panicle green
F7	Seeds milky ripe/ panicle green
F8	Seeds dough ripe/ beginning panicle coloration
F9	Seeds physiological ripe / panicle fully coloured

$RGR = LWR \times SLA \times NAR$. NAR is the net assimilation rate (g m^{-2} day^{-1}), LWR is leaf weight ratio (g g^{-1}), and SLA is the specific leaf area (m^2 kg^{-1}). SLA was calculated as the amount of leaf area per unit of leaf dry weight, LWR as the leaf fraction of the total dry plant biomass, and RGR as the natural logarithm of the relative increase in plant biomass over the mentioned period of time: $RGR = \ln(W_2/W_1)/(t_2\text{-}t_1)$ (Lambers and Poorter, 1992). After physiological ripening, seed yield was measured as dry seed weight per plant, thousand-seed weight (TSW) was recorded using a seed counter (Contador, Pfeuffer GmbH, Jefferson, OR, USA) and harvest index (HI) was calculated as the ratio of dry seed weight and dry aboveground biomass. The salt tolerance index (STI) was calculated as the ratio of dry biomass (above-ground biomass or seed yield) of salt-treated plants and the dry biomass of control (0 mM NaCl) plants.

2.4. Assessment of physiological traits

Several physiological traits were measured during the growing season. Stomatal conductance (*gs*) was measured in the second fully developed non-shadowed leaf using a portable leaf porometer (Decagon Devices Inc., WA, Australia) throughout the growth cycle between 10:00 − 12:00 h on a sunny day, unless specified otherwise. Leaf chlorophyll content was measured using a SPAD 502 Meter (Minolta, Osaka, Japan) on the second fully developed leaf. The maximum photochemical efficiency of photosystem II after dark adaptation (Fv/Fm) was measured on the same leaf, between 10:00 − 12:00 h using a OS/30 P portable fluorometer (Optics-Science Inc., USA). Relative water content was calculated at the onset of flowering as $RWC = \frac{(FW-DW)}{(TW-DW)} * 100\%$, where TW is the turgid weight, FW is the fresh weight and DW is the dry weight of an entire single young leaf. Turgid weight was determined after the leaf was imbibed in ultrapure water (Milli-Q®) in the dark for 12 h.

2.5. Ion content measurements

The ion contents in leaves, stems, roots and bladder cells were measured using Ion Chromatography (IC) system 850 Professional (Metrohm Switzerland). For this purpose, oven-dried tissues were ground to fine powder using a hammer mill with 1 mm sieve. Twenty-five mg per sample was turned into ash in a furnace at 550 °C for 5 h. Ten ml of Milli-Q® water was added to the ashes and these were shaken for 15 min at 5000 rpm at 100 °C. Prior to injection onto the IC system, samples of leaves, stems and roots were diluted 400 times with Milli-Q®. Nitrate was also measured using Ion Chromatography but the samples were prepared differently. Forty mg of grinded dry leaves was weighed in a glass screw cap tube. Five ml of Milli-Q® was added to the sample and this was mixed by vortexing for 5 min. After shaking, the samples were heated in an ultrasonic bath for 30 min at 80 °C. The samples were transferred to a thermomixer and incubated for 1 h at 5000 rpm at 100 °C. After cooling down, samples were centrifuged for 5 min at 4200 rpm and diluted 50 times previous the injection to the IC column. Ion contents were calculated as the amount of ions per unit of dry weight (mg ion g^{-1} dry mass) and the ion concentrations were estimated based on the water content of the tissue. The ratio K^+/Na^+ was calculated based on mg K^+/ mg Na^+ content.

2.6. Characterization of epidermal bladder cells (EBCs)

A dedicated experiment was conducted in order to obtain enough epidermal bladder cells to evaluate their potential function as deposits of salt ions during salt stress in quinoa. Plants of the cultivar Pasto were grown either in control conditions or with a salt concentration of 250 mM NaCl. After eight weeks of treatment, 200 leaves were collected from control and treated plants. EBCs were brushed from the abaxial and adaxial sides of half of the leaves. The fresh weight of the 100 intact leaves, 100 leaves after removing the bladders, and the brushed bladders was recorded and the leaf area was measured as described before. The ion content in the leaves and in the EBCs was measured as previously described, but the EBCs were reduced to ashes and weighed without the grinding step during the sample preparation. The total biomass of both leaves and EBCs was decomposed as follows: fresh weight is the total biomass; FW = W ash + W water + W organic matter; W water = FW – DW; and W organic matter = DW – W ash.

2.7. Statistical analysis

General analyses of variance (ANOVA) were performed to determine the significance of genotypic differences, salt treatment differences and their interactions ($p < 0.05$). The analyses were performed following a standard procedure for a linear mixed model, for which genotype and salt treatment were considered fixed effects and blocks random effects. The above-mentioned model was: $y_{ijk} = \mu + b_k + \alpha_i + d_{ik} + \beta_j + \alpha\beta_{ij} + e_{ijk}$, were y_{ijk} is the response variable, μ is the grand mean, α_i is the salt treatment effect, β_j is the genotype effect, $\alpha\beta_{ij}$ is the genotype-by-salt interaction effect, b_k and d_{ik} are the block effects and e_{ijk} is the residual error. Multiple comparison analyses were performed using Fisher's protected least significant difference (LSD) test on genotype means. All statistical analyses were performed using the software Genstat 19th Edition (VSN International Hemel Hempstead, UK).

3. Results

3.1. Experiment 1: Full plant cycle response of European sweet quinoa to a wide range of salinity levels

3.1.1. Overall performance

Four sweet quinoa genotypes: Atlas, Jessie, Pasto and selRiobamba were grown at four salt concentrations: 0, 100, 200, and 300 mM NaCl. Plant biomass was decreased significantly already at the time of the first destructive harvest (11 weeks after sowing, 6 weeks of treatment) when plants started to flower (Fig. 1A), but the mean reduction was only 5 % at 100 mM NaCl, while it reached 43 % at 300 mM NaCl. The averaged salt tolerance index (STI) at the onset of the flowering was 0.96 at 100 mM, 0.79 at 200 mM and 0.62 at 300 mM NaCl (Fig. 1B). Interestingly, until this stage, Jessie and selRiobamba had higher aboveground biomass at 100 mM NaCl than under control conditions.

The effect of salt on seed yield was examined at the end of the growing cycle (Fig. 1C). There was significant variation between genotypes and treatments, but not for the interaction between both. SelRiobamba was the variety with the highest yield, followed by Atlas and Pasto, and Jessie with the lowest yield. The average salt-induced seed yield reduction for the cultivars was 29 % at 100 mM, 57 % at 200 mM and 65 % at 300 mM NaCl. SelRiobamba and Pasto had the lowest yield reduction (25 %) at 100 mM NaCl salinity. At the most severe salinity treatment (300 mM NaCl) selRiobamba remained the least affected variety (60 % reduction) but Pasto was more affected than Jessie (68 %). Significant genotypic variation ($p < 0.001$) was detected for Harvest Index (HI) (Fig. 1D), but surprisingly, HI was not significantly affected by the salt treatment. On average, the harvest index was reduced by only 4 % at 100 mM and by 20 % at 300 mM NaCl. In fact, it was the least affected parameter by salinity, which reflects the halophytic property of quinoa to still be able to allocate carbon to seeds even when exposed to high salinity levels.

3.1.2. Ion contents

Ion contents in the leaves were measured at the onset of flowering of the plants (6 weeks of salt treatment). Shoot Na^+ and Cl^- concentrations increased significantly in plants under all salt treatments, with Cl^- increasing much more than Na^+ (Fig. 2A-B). Jessie had the highest

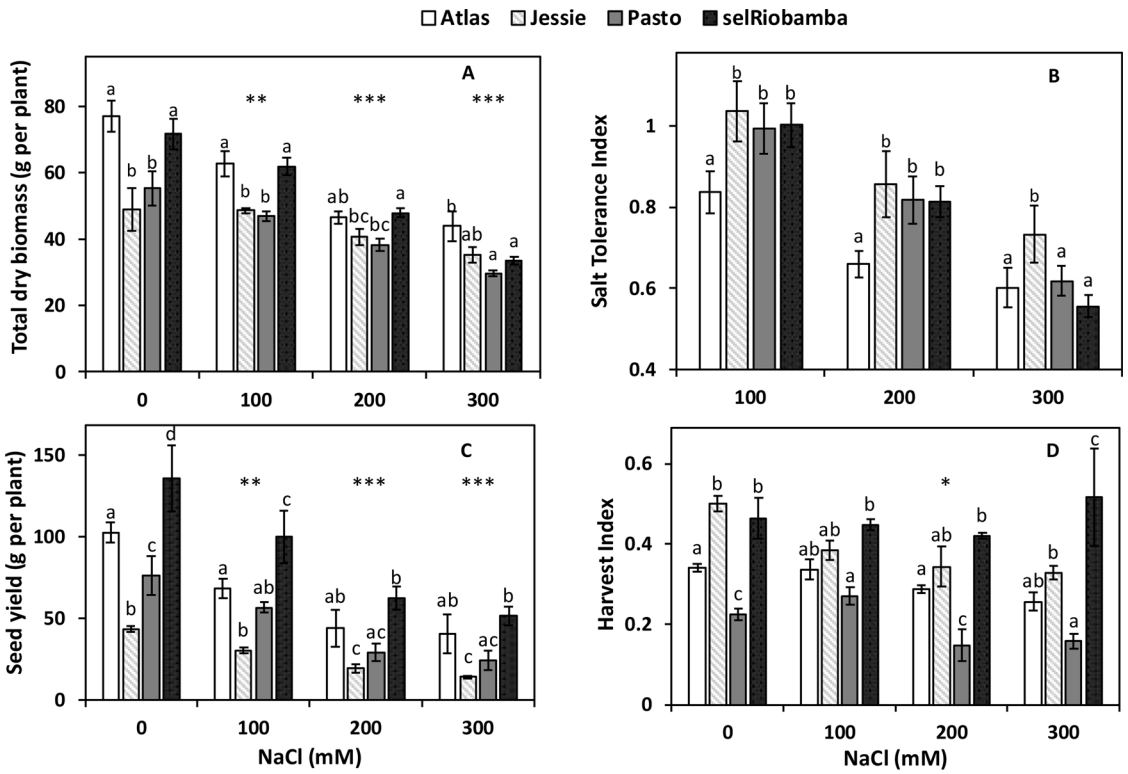

Fig. 1. Agronomic characteristics of quinoa plants grown in Experiment 1 at various salinity levels. A) Total dry biomass weight 11 weeks after sowing, 6 weeks after salt application at the start of flowering. B) Salt tolerance index calculated as above biomass DW treatment/ above biomass DW control. C) Seed yield. D) Harvest index. Means of 5 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between varieties (within each salt treatment) are shown with different letters. Asterisks denote statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between the salt treatments and the control.

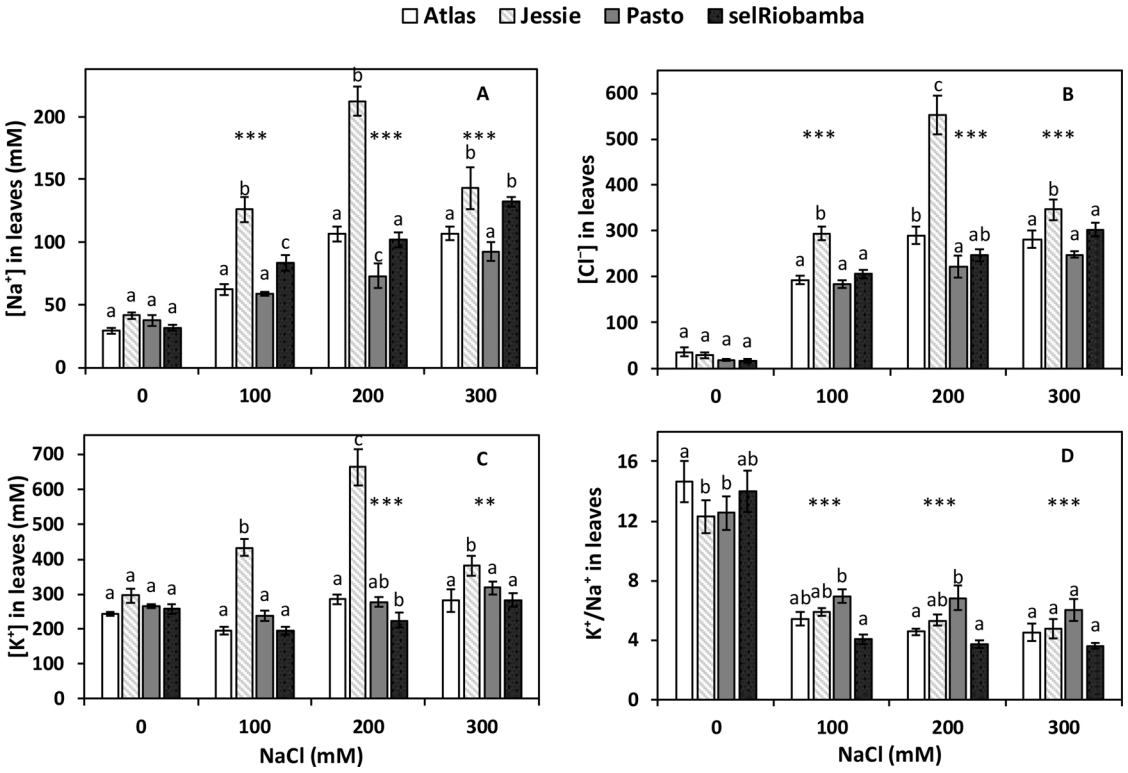

Fig. 2. Ion contents in young leaves of plants grown in Experiment 1, 11 weeks after sowing, 6 weeks after salt application. A) $[Na^+]$. B) $[Cl^-]$. C) $[K^+]$. D) K^+/Na^+. Means of 5 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between varieties (within each salt treatment) are shown with different letters. Asterisks denote statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between the salt treatments and the control.

accumulation of Na^+ and Cl^- at all salt levels. Remarkably, the highest levels of shoot Na^+ and Cl^- were not detected under the most severe 300 mM NaCl salt treatment. At 200 mM NaCl, shoot Na^+ reached the maximum concentration of 213 mM and Cl^- of 553 mM. Pasto displayed the lowest Na^+ and Cl^- concentrations in all the treatments. K^+ concentrations in the leaves were increased in all the salt treatments. Jessie had the highest shoot [K^+] under salinity, followed by Pasto (Fig. 2C). The latter had also the lowest levels of Na^+ and Cl^-; as a result, it had the highest K^+/Na^+ ratio in all the treatments, with the lowest value (6) at the most severe salt stress (Fig. 2D).

3.2. Experiment 2: Full plant cycle response of quinoa cultivars to extreme salinity

We further evaluated the impact of extreme salinity on quinoa in Experiment 2. Plants were treated with irrigation solutions containing 300 or 400 mM NaCl. The soil substrate (vermiculite nr 1) used in this experiment had a very high water-holding capacity and minimal drainage, and in order to prevent anoxia of the roots, the frequency of irrigations for the treated plants had to be lowered to once every ten days, leading to gradual accumulation of salt in the pots. At the end of the season, the EC of the 300 mM NaCl − treated pots reached ~55 dS/m, and the 400 mM treatment reached 65 dS/m. The salinity level applied in this experiment was therefore substantially higher than in the first experiment, and exceeded the maximum salinity level at which quinoa was reported to still produce grain (Razzaghi et al., 2015). To facilitate comparison between experiments and treatment levels, the 300 mM NaCl irrigation treatment will be further referred to as 55 dS/m treatment and the 400 mM NaCl as 65 dS/m treatment.

At the first destructive harvest 6 weeks after salt application (onset of flowering) Atlas had the highest biomass under control conditions (63 g/plant), followed by Red Carina, selRiobamba, Jessie and Pasto (Fig. 3A). At this time, the salt treatments already had a considerable effect on the total dry biomass of all the varieties, but the difference in biomass between the two salt treatments was small. The total biomass mean was 17 g per plant for the 55 dS/m treatment and 15 g per plant for the 65 dS/m treated plants and this resulted in a mean biomass-based salt tolerance index of 0.34 at 55 dS/m NaCl irrigation and 0.27 at 65 dS/m (Fig. 3B). As shown in Fig. 3C, seed yield of the cultivars was reduced by 95 % at 55 dS/m and by 97 % at 65 dS/m NaCl. Despite the strong reduction in biomass, all the plants survived and produced seed.

Ion concentrations in young leaves were quite different between the two salt treatments (Fig. 4). Curiously, Na^+ and Cl^- concentrations were higher under 55 dS/m compared to the 65 dS/m treatment. Mean [Na^+] was 48 % higher at 55 dS/m than at 65 dS/m and [Cl^-] was 28 % higher. Interestingly, [K^+] was also increased (by 35 %) under the severest salinity stress (EC of 65 dS/m) compared to control.

3.3. Experiment 3: Detailed evaluation of the growth, ion dynamics and physiology of quinoa exposed to high salinity (400 mM NaCl)

3.3.1. Biomass under high salinity

In Experiment 3, plant height under control and highly saline conditions (400 mM NaCl) was measured weekly during the whole growing season (Fig. 5A). The height of plants started to be affected two weeks after salt application and this difference became significant one week later. From this time point onwards, the control plants increased their height until eight weeks after the start of the salt treatment, while the height of the salt-treated plants increased at a lower rate and stopped increasing earlier. At the end of the season, this resulted in an average 40 % lower plant height at 400 mM NaCl compared to 0 mM (average for the three cultivars). Destructive harvests throughout the whole crop cycle allowed monitoring the effect of salinity on the biomass of plants at different stages of development. Four weeks after the start of the salt treatment, the STI (for shoot dry biomass) at 400 mM salt was only 50

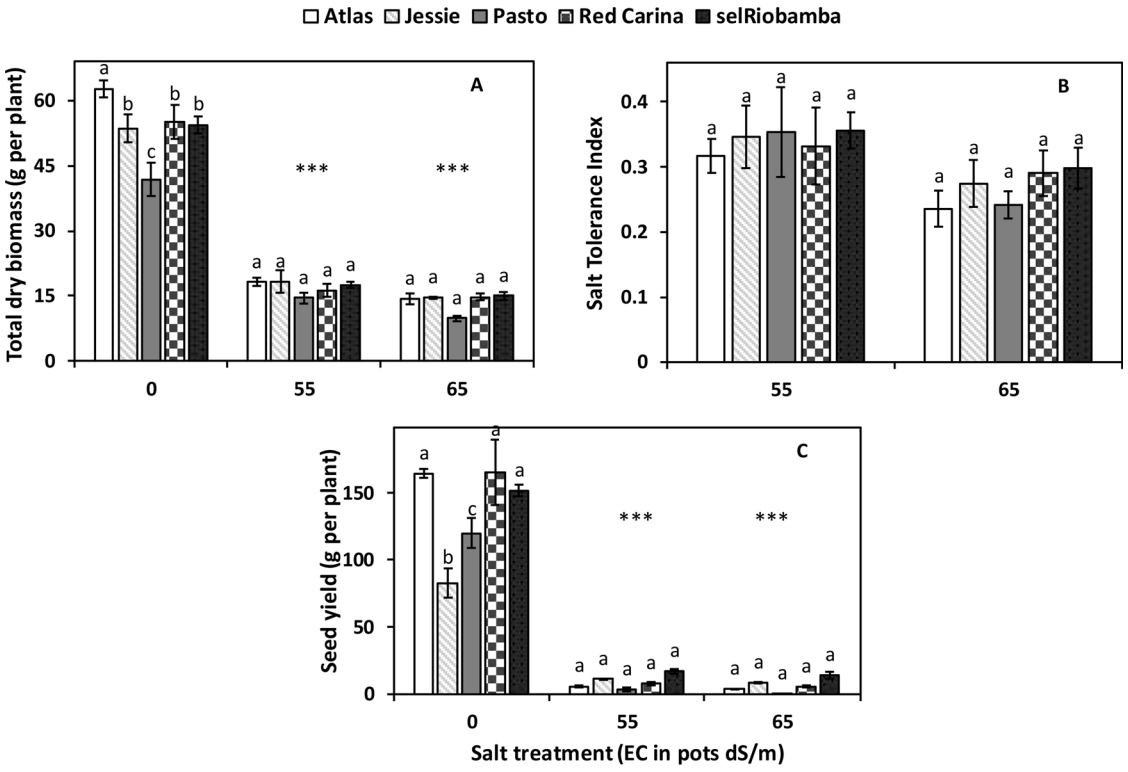

Fig. 3. Agronomic characteristics of quinoa plants grown in Experiment 2 at high concentrations of salt. A) Total dry biomass weight 11 weeks after sowing, 6 weeks after salt application. B) Salt tolerance index calculated as above biomass DW treatment/ above biomass DW control. C) Seed yield. Means of 4 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between varieties (within each salt treatment) are shown with different letters. Asterisks denote statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between the salt treatments and the control.

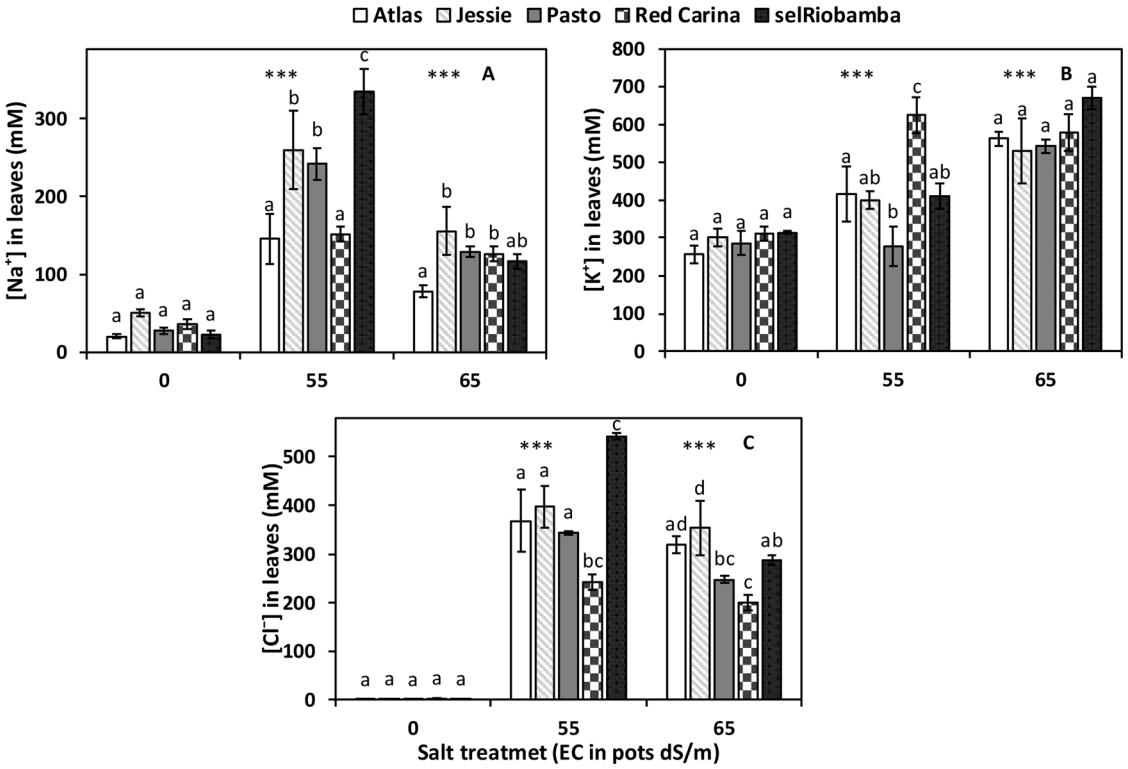

Fig. 4. Ion contents in young leaves of plants grown in Experiment 2 at high concentrations of salt (11 weeks after sowing, 6 weeks after salt application). A) [Na^+]. B) [K^+]. C) K^+/Na^+. Means of 4 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between varieties (within each salt treatment) are shown with different letters. Asterisks denote statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between the salt treatments and the control.

% and the effect of salt increased strongly with time. After ten weeks of salt treatment Jessie had the highest STI (31 %), followed by selRiobamba (26 %) and Pasto (20 %). This differential response was stronger for the STI based on seed yield at the end of the season. Jessie had the highest seed-based STI of 19 %, followed by selRiobamba with 13 % and Pasto had the lowest of only 2 %: Pasto survived well (small but green plants), but hardly produced grain (Fig. 5B). Yield parameters including thousand seed weight (TSW) and Harvest Index (HI) are shown in Fig. 6A-C. Similar to Experiment 2, seed yield was strongly compromised at 400 mM NaCl salinity (average reduction of 88 %). TSW was reduced for all varieties (average mean reduction 34 %) with Pasto having the lowest values. Remarkably, the harvest index of selRiobamba and Jessie was not significantly influenced by the salt treatment. Pasto's harvest index however was strongly reduced by 83 %.

3.3.2. *Effect of high salinity on ion dynamics*

3.3.2.1. Roots. After 4 weeks of salt stress, the concentrations of Na^+ and Cl^- in the roots were increased in all varieties (mean [Na^+]: 62 mM and [Cl^-]: 42 mM, compared to 4 mM and below the detection level, respectively, under control conditions), but still much lower than the 400 mM NaCl concentration in the root medium. While no significant differences were observed between cultivars at the first time point, after seven weeks of the salt treatment the root [Na^+] was higher than that of the root medium for Jessie and selRiobamba (452 mM and 517 mM, respectively) but not for Pasto (370 mM). Root [Cl^-] was lower than that of Na^+ and remained lower than in the root medium (mean = 243 mM). Pasto had the lowest root accumulation of Na^+ and Cl^- in the first seven weeks of treatment, but these were higher and close to those of the other cultivars at the last measured time point, 10 weeks after the start of salt application (415 and 253 mM, respectively) (Fig. 7A-B).

The [K^+] in roots of plants grown at 400 mM NaCl was relatively stable throughout the season. Among the varieties, Pasto always had the highest [K^+]. Pasto was also the only variety with higher [K^+] under salt stress compared to the control after four weeks of salt treatment. For all varieties, the K^+/Na^+ ratio in the salt-treated roots was already reduced at four weeks after the start of the salt treatment and remained lower than 1.0 throughout the season. In comparison, in roots of control plants this index was always above 4 (Fig. 7C-D).

3.3.2.2. Leaves. Ion concentrations were measured in young and old leaves separately. No significant differences were found between both (Figure S1). The ion contents of the young leaves are presented in the following section, but these are representative of total leaves ion contents.

Na^+ and Cl^- accumulated in the leaves of salt-stressed plants over time, but in contrast to the roots, [Cl^-] was higher than [Na^+] in all varieties at all time points (Fig. 7E-F). For the first two time points, Pasto had the lowest Na^+ and Cl^- concentrations in the leaves. After ten weeks of treatment, the concentration of both ions reached similar and very high values in all three cultivars; the average [Na^+] was 667 mM and the average [Cl^-] was 755 mM. Remarkably, the salt treatment also caused an increase in [K^+] in leaves at all the time points and varieties (Fig. 7G). Consequently, the K^+/Na^+ decreased but remained relatively high throughout the growing season (Fig. 7H), with an average of 1.5 for the three varieties after 10 weeks of stress. Pasto had the highest shoot [K^+] and lowest [Na^+] and therefore the highest K^+/Na^+ in the shoot.

Fig. 8A-C depicts the distribution of a number of inorganic anions and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-}, SO_4^{2-}, NO_3^-) over roots, stems and young leaves seven weeks after the start of salt treatment. The concentration of Cl^- in salt-treated plants showed an increasing gradient from root to stem to leaves (mean [Cl^-]: 243 mM in roots, 396 mM in stems and 580 mM in leaves). Na^+ accumulation in leaves was much lower than Cl^- accumulation, and lower than Na^+

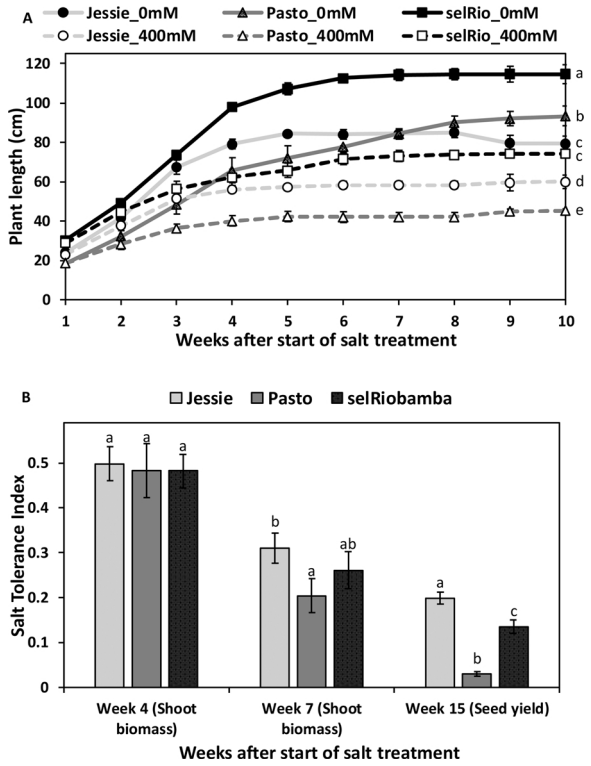

Fig. 5. Growth responses of quinoa growing at 400 mM NaCl in Experiment 3. A) Plant height development through the season. Means of three plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between any variety and salt treatment combination are shown with different letters. B) Salt tolerance index in different harvests through the season based on biomass of plants. Means of 3 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between varieties for each time point are shown with different letters.

accumulation in the other two tissues (448 mM in roots, 525 mM in stems and 369 mM in leaves), suggesting an active exclusion of Na^+ from leaves. Root [K^+] was reduced by the NaCl treatment, while in the stem [K^+] was 25 % higher in treated plants compared to the controls, and in the leaves it was increased by 133 %. Similar to K^+, Mg^{2+} and phosphate concentrations were reduced in the roots, but not in the leaves. The concentration of Ca^{2+} was reduced by salinity in all the tissues. Sulphate was the least affected ion by the salt treatment. The concentration of nitrate was measured only in young leaves (Figure S2). Salt treatment caused a reduction of the nitrate content that substantially differed between varieties. Leaf nitrate in Pasto was hardly affected (7 % reduction), while selRiobamba had the highest reduction of 73 %. Interestingly, the electrical balance of inorganic ions was positive to a similar degree for both salt concentrations. This might imply that no additional energy is required for the synthesis of negative organic compounds under salt stress to restore the electrical neutrality.

3.3.3. High salinity stress and plant physiology

All the salt-treated plants showed significantly lower leaf stomatal conductance (*gs*) compared to control plants. However, the effect of salt on *gs* was only detected after two weeks of salt application (25 % reduction of *gs*). Three weeks after the beginning of the treatment the effect of salinity on stomatal conductance became considerably more pronounced, with an average reduction of 60 %. Stomatal conductance values remained low during the rest of the growing period, with hardly any fluctuations due to weather conditions or physiological maturation. The highest *gs* reduction was found in Pasto, followed by selRiobamba and Jessie (Fig. 9A). Stomatal conductance was also measured five times over a 24 h timespan (Fig. 9B), at the onset of flowering (seven weeks after salt application). Under control conditions, daily *gs* was characteristic of a C3 crop during a summer day (middle of June) in the Northern Hemisphere. Sunrise occurred around 5:00 AM and this coincided with an increase in *gs* after very low levels during the night. During the day, *gs* increased reaching its maximum value around 3:00 PM, after which it declined and totally stopped after sunset (9:00 PM). The *gs* peak was also observed around 3:00 PM for stressed plants, but the conductance declined faster and was below the detection threshold of the porometer many hours before sunset. Hence, not only was the

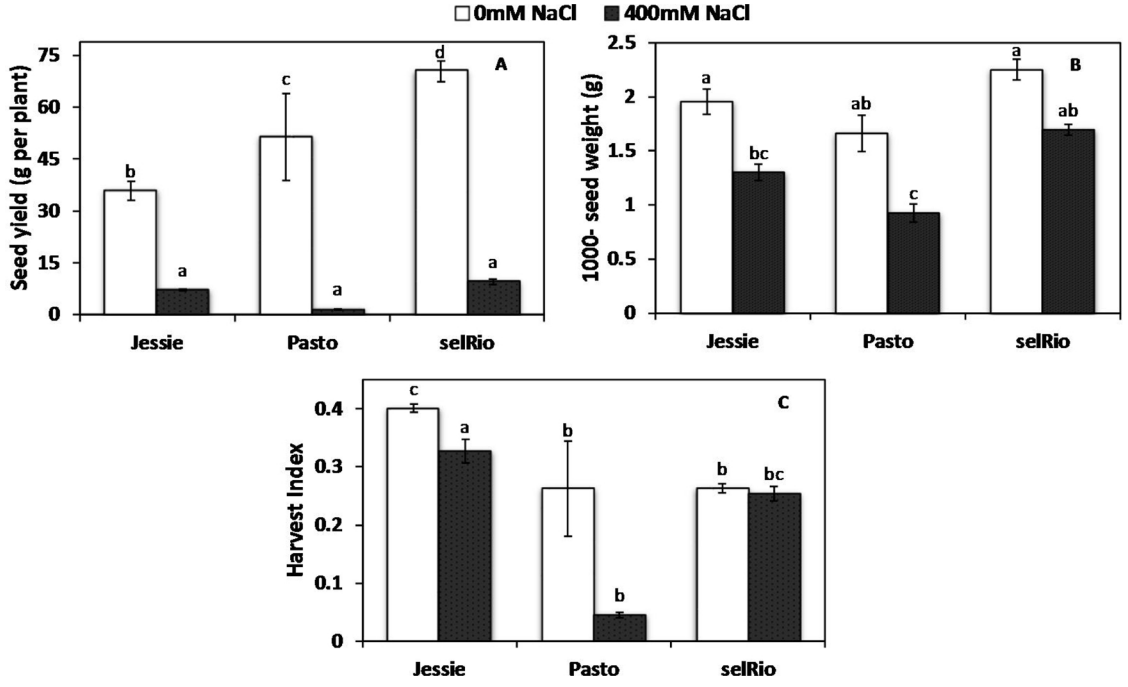

Fig. 6. Yield traits of quinoa growing at 400 mM NaCl in Experiment 3. A) Seed yield. B) Thousand-seed weight. C) Harvest index. Means of 3 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between any variety and salt treatment combination are shown with different letters.

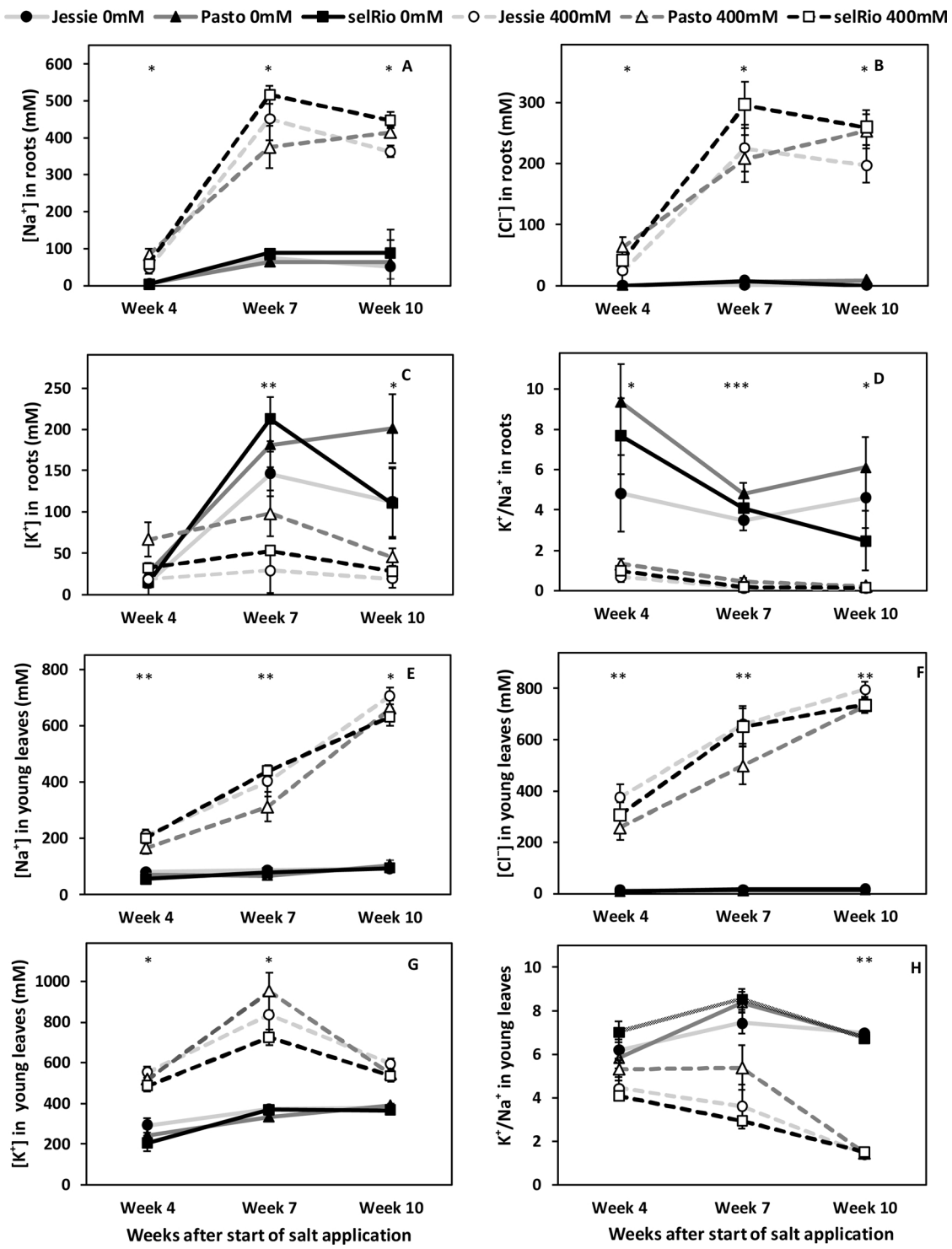

Fig. 7. Na^+, K^+, Cl^- contents and K^+/Na^+ ratio in roots and young leaves measured throughout the growing season in plants grown in Experiment 3. Means of 3 plants. Error bars indicate SE of individual means. Asterisks denote statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between the salt treatments and the control.

maximal *gs* decreased under salinity stress, but the time that stomata were opened during the day was also shortened. Maximum quantum yield of PSII (Fv/Fm) of quinoa leaves was not affected by severe salt stress (average value for control and salt treated plants was 0.79) (Figure S3). Chlorophyll content was measured throughout the growing season and was significantly influenced by salinity as well as genotype (Fig. 9C). At the beginning of the treatment (one week after salt application) the chlorophyll content was higher in the salt treated plants. It remained higher throughout the whole season for Pasto, while for Jessie and selRiobamba it was reduced by salt after five weeks of salt treatment. Leaf RWC was significantly reduced (25 %) by salinity (Fig. 9D).

3.3.4. High salinity stress and growth

The mean RGR for all the cultivars was 0.111 d^{-1} at 0 mM NaCl and 0.0985 d^{-1} at 400 mM NaCl (Fig. 10A). The difference in the leaf

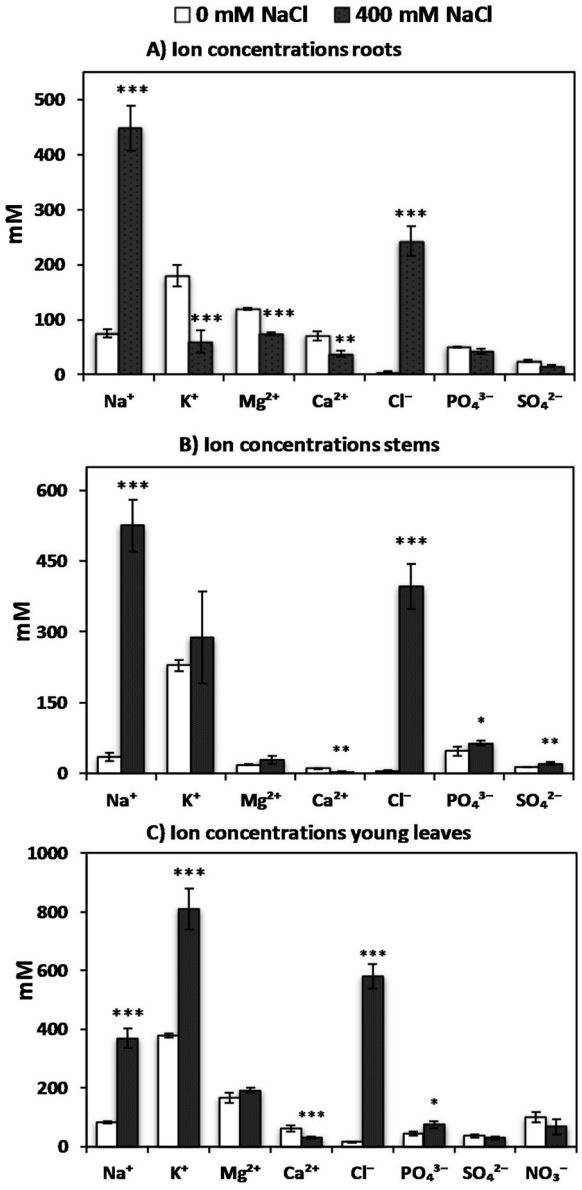

Fig. 8. Ion contents in different tissues of quinoa grown in Experiment 3 at 400 mM NaCl 12 weeks after sowing, 7 weeks after start of the salt treatment. A) Roots. B) Stems. C) Young leaves. Means of three varieties (Jessie, Pasto, selRiobamba) and 3 replicates per variety. Error bars indicate SE of individual means. Asterisks denote statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between the salt treatment and the control for each ion content.

weight ratio (LWR) between treatments or cultivars was not significant (Fig. 10B). The most significant effect of the salt treatment on RGR components was a decrease in the specific leaf area (SLA), from 307 to 206 $m^2\ kg^{-1}$ for 0 and 400 mM NaCl treatment, respectively (Fig. 10C). The physiological component of RGR, net assimilation rate (NAR), was surprisingly increased under saline conditions, from an average 13 g $m^{-2}\ d^{-1}$ in control plants to 16 g $m^{-2}\ d^{-1}$ in salt treated plants (Fig. 10D).

3.4. Contribution of epidermal bladder cells to salt tolerance

Salt stress reduced the water content of epidermal bladder cells (Fig. 11A). We measured the content of Na^+, Cl^- and K^+ in 1) young leaves including EBCs, 2) young leaves after the removal of EBCs, and 3) brushed EBCs (Fig. 11B). The results were expressed as the amount of ions (mmol) per area of leaves (m^2), or in the EBCs removed from the same leaf area. This allowed the assessment of the relative contribution of EBCs to ion storage compared to the total amount of ions accumulated in the leaves. The concentration of Na^+, Cl^- and K^+ in the bladders was higher in the salt-treated plants compared to the controls (Fig. 11B). However, the percentage of ions accumulated in the EBCs relative to the total leaf ion content was only 5.4 % for Na^+, 6.5 % for Cl^- and 15 % for K^+. The relatively high accumulation of K^+ in the bladders coincides with the high levels of this ion distributed in all the leaf tissue. Based on the results in our study, storage of salt in EBCs is not likely to contribute significantly to reduce levels of Na^+ and Cl^- in the leaves.

4. Discussion

To fully understand why quinoa can survive and reproduce in highly saline conditions while being an economically productive food crop under normal, non-saline conditions, it is essential to gain insight in the physiological changes and adaptations during the crop cycle under prolonged exposure to high salt levels in the soil. Our study demonstrates that quinoa varieties utilize salt exclusion strategies to produce relatively high yields under mild salinity or short-term stress, while tissue tolerance mechanisms enable the plants to survive and even reproduce under severe and prolonged salinity.

4.1. Ion and water dynamics throughout the growing season

Given the importance of water availability for all aspects of plant physiology, plants suffer from salinity first and foremost because of the problems with water uptake. Water uptake from the root medium is a complex process mediated by long-distance shoot-to-root signals. Under saline conditions, water uptake and transport in the plant is also influenced by Na^+ and Cl^- uptake and distribution over the plant tissues. In a two-step model, Na^+ passively enters root cells via non-selective cation channels driven by a negative membrane potential and a low $[Na^+]$ in the cytosol (Britto and Kronzucker, 2015). According to this model, sodium ions rapidly exit the cells through the SOS1 transporter, the only cytoplasmic Na^+ efflux transporter identified until now. By monitoring the ion contents in different tissues throughout the growth of the plants we were able to identify specific, time- and stress level-dependent strategies used by quinoa to cope with salt stress. The complex ions dynamics described for quinoa is likely to be associated to the activity of key, possibly novel, ion transporters. The expression of only a few transporters (SOS1, NHX) has been examined in short-term experiments in quinoa and variable responses between varieties have been reported (Maughan et al., 2009; Ruiz-Carrasco et al., 2011; Schmöckel et al., 2017). An *in silico* exploration of ion transporters annotated in the genome of quinoa (Jarvis et al., 2017) revealed a high diversity and abundance of K^+, Na^+ and Cl^- transporters in quinoa compared to other species reported in literature (Lebaudy et al., 2007; Véry et al., 2014). A comprehensive study of the expression of these transporter families in different tissues at different developmental stages of the crop would help to elucidate the role of the ion transporters involved in the different salt tolerant strategies reported here, but such study is beyond the scope of this manuscript.

In an early stage of development (budding) and relatively short exposure to high salt stress (4 weeks of 400 mM NaCl) the concentration of Na^+ and Cl^- in the roots was considerably lower than in the soil medium (Fig. 12, upper panel), suggesting that the plants actively excluded the ions from the roots. This may represent a substantial challenge to the plants: the water needs to be taken up by the roots against an ion gradient. Stomatal conductance in our study was not significantly reduced until three weeks after the stress was imposed, indicating that the quinoa varieties were not saving water but maintained high photosynthesis and growth rates during the first two weeks of exposure to high salinity. This implies that during this first stage of salt

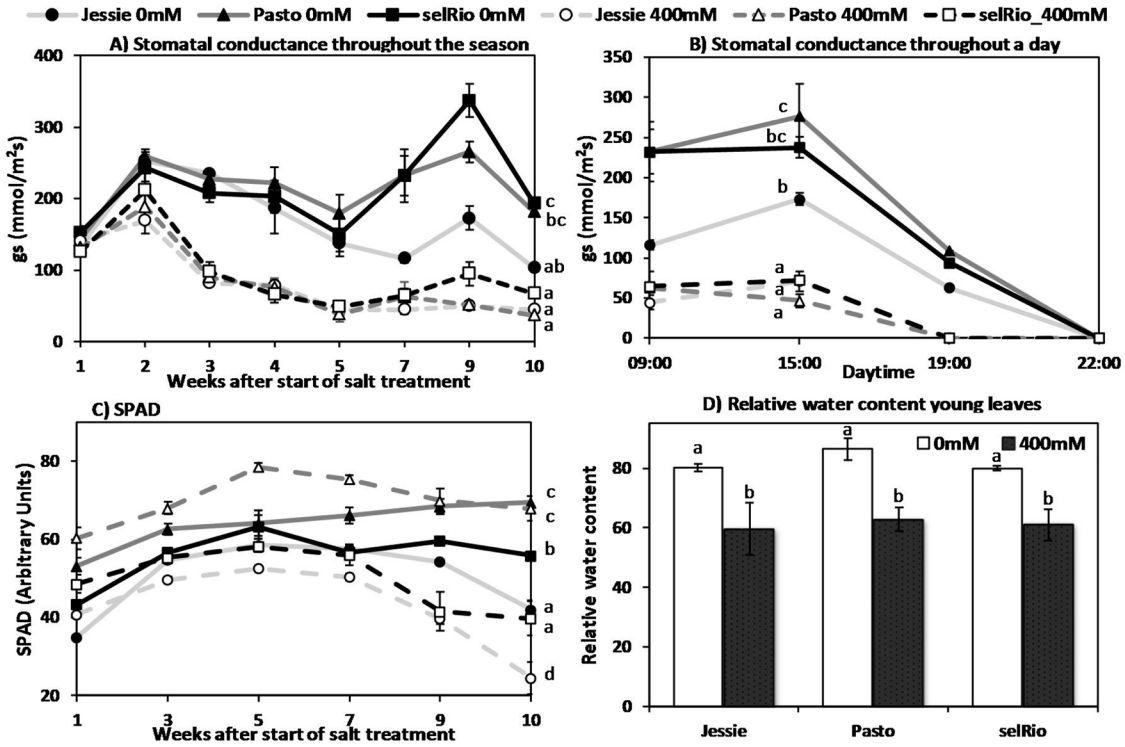

Fig. 9. Physiological traits measured in quinoa grown at 400 mM NaCl in Experiment 3. A) Stomatal conductance through the growing season measured from 9:00 AM until 11:00 AM. B) Circadian variation of stomatal conductance. C) Chlorophyll content (SPAD measurements) through the growing season. D) Relative water content. Measurements depicted on B and D were taken 7 weeks after the start of salt treatment. Means of 3 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between any variety and salt treatment combination are shown with different letters.

stress, quinoa plants were still able to take up water from the root medium, which suggests that osmotic adjustment most likely relied on the production of organic osmolytes. The contribution of these organic osmolytes for osmotic adjustment in quinoa was previously reported (Shabala et al., 2012).

Our results indicate that at flowering (7 weeks after beginning of the stress), the plants have changed strategies (Fig. 12, middle panel). At this time, [Na^+] in the roots equalled that of the root medium (400

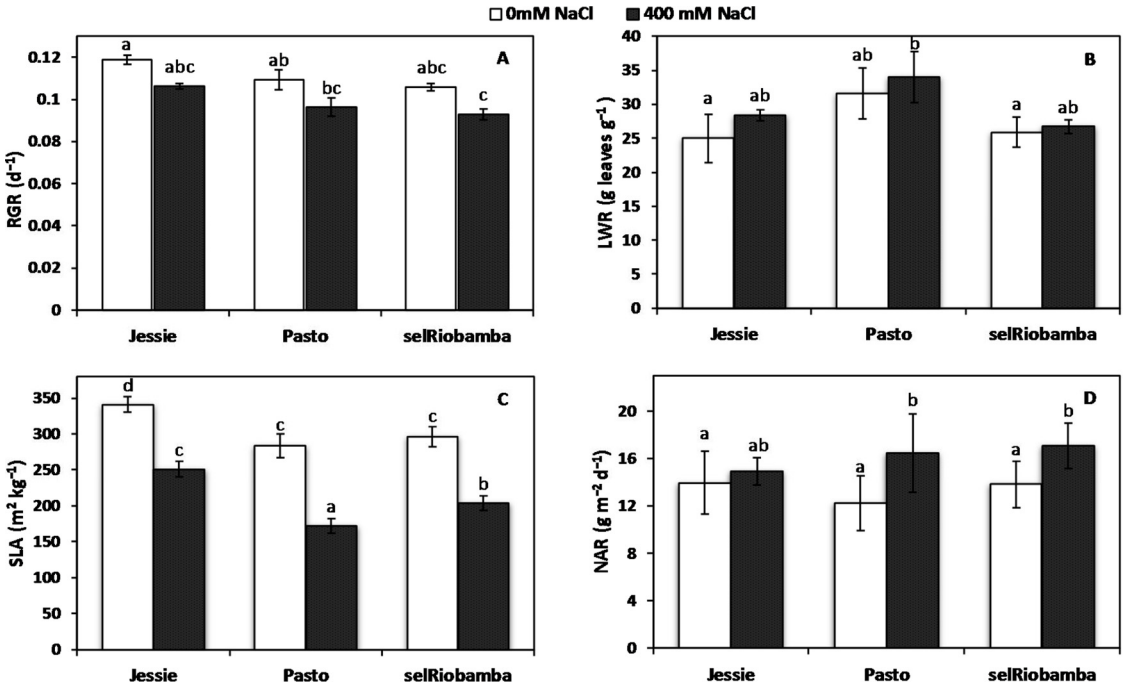

Fig. 10. Effect of severe salt stress (400 mM NaCl) on Relative Growth Rate components of plants grown in Experiment 3 from the transplanting date (3 weeks after sowing) until 4 weeks after beginning of salt treatment (9 weeks after sowing). A) Relative growth rate (RGR); B) Leaf weight ratio (LWR); C) Specific leaf area (SLA); D) Net assimilation rate (NAR). Means of 3 plants. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between any variety and salt treatment combination are shown with different letters.

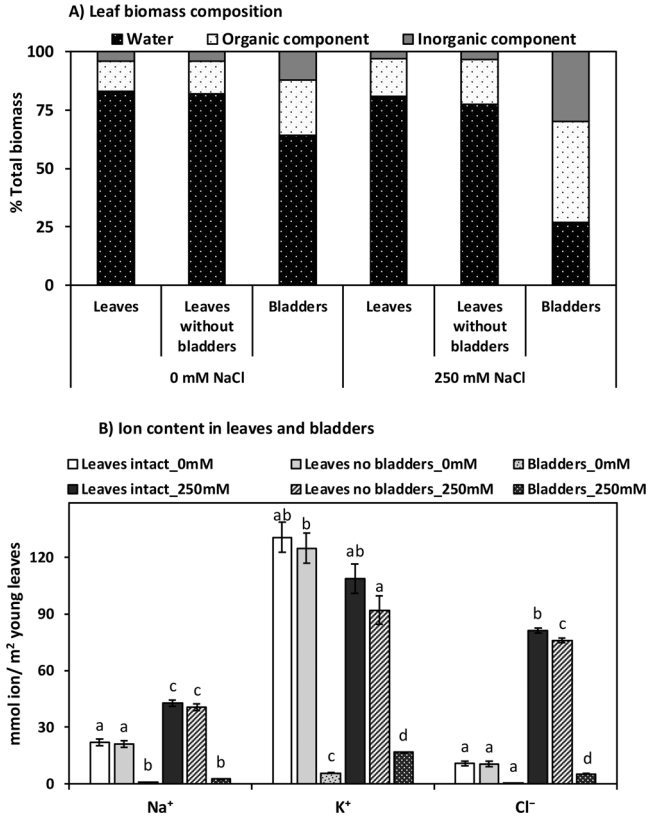

Fig. 11. Epidermal bladder cells (EBCs) composition in quinoa plants (variety Pasto) grown at 250 mM NaCl. A) Leaf and bladders biomass composition under control and stress conditions. B) Na^+, K^+ and Cl^- contents in leaves and bladders presented as the content of ions (mmol) per m^2 leaf area. Means of 5 biological replicates. Error bars indicate SE of individual means. Statistically significant differences ($p \leq 0.05$) between any tissue and salt treatment combination (for each specific ion) are shown with different letters.

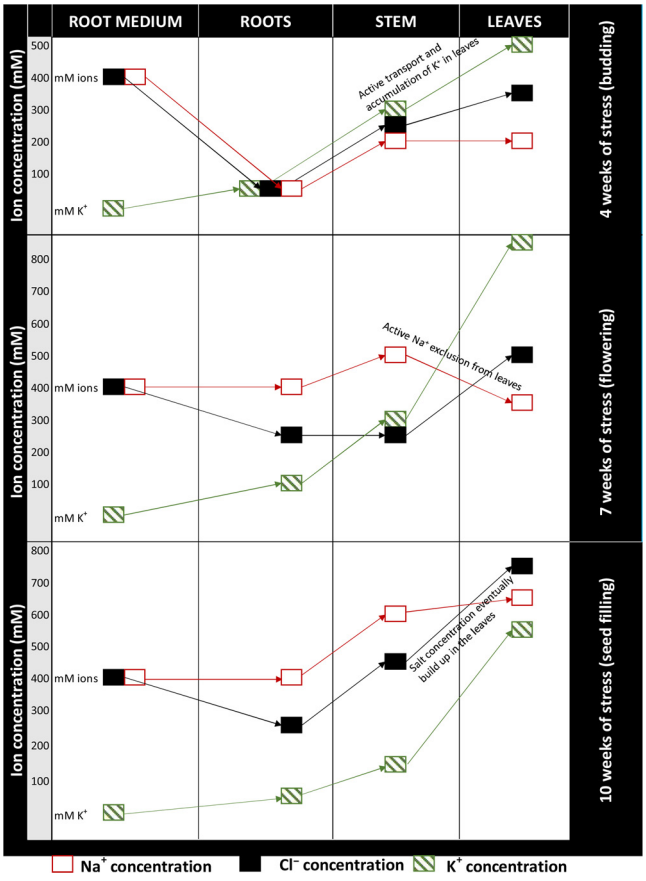

Fig. 12. Schematic representation of ion dynamics in quinoa throughout the growth cycle based on ion contents measurements in different tissues.

mM), while the Cl^- was still lower (250 mM), and remained like that until the end of the season. The increased [Na^+] in the root tissues helps to restore the osmotic balance with the root environment, facilitating water uptake. However, at this stage, stomatal conductance was considerably lower than early in the season. Previous reports showed that quinoa reduced transpiration under salt stress at similar stages of development and stress levels (Adolf et al., 2012; Orsini et al., 2011). Decreased transpiration will reduce the rate of Na^+ and Cl^- accumulation the leaves. Munns et al. (2020) reported that most plant species are able to exclude about 98 % of the salt in the root medium, but in spite of this the salt concentrations in the shoot will still be equal to that of the root medium after 3–4 weeks. In most of our varieties, the shoot Na^+ and Cl^- concentrations were lower than the root medium at 4 weeks, suggesting a high level of root Na^+ exclusion in quinoa, but at 7 weeks a similar concentration than the root media was reached. [Na^+] in the shoot of the variety Pasto however was still lower. From this stage onwards, quinoa appears to mostly rely on tissue tolerance to cope with salinity. High levels of Na^+ and Cl^- in the cytoplasm are detrimental for cells (Maathuis et al., 2014); therefore, vacuolar compartmentalization of these ions is a likely strategy in quinoa at prolonged and high levels of salinity. The sequestration of Na^+ and Cl^- in the vacuole not only protects the cytoplasm against toxicity, but also increases the osmotic potential of the cell in a cost-effective manner, as long as the cytosolic osmotic potential is adjusted accordingly. Our results supports previous indications that K^+ may play an important role in this adjustment (Rubio et al., 2020). While in the roots K^+ concentration was decreased in stressed plants compared to controls from the earliest time point measured until harvest, it was higher in young leaves of salt-stressed plants than in control leaves and even higher than the [Na^+]. In our experiments, quinoa appeared to be able to maintain leaf cell turgor (no signs of wilting) even when leaf RWC was reduced, which is indicative of a strategy of osmotic adjustment (Negrão et al., 2017).

Towards the end of the growing cycle (seed filling, 10 weeks after stress application) Na^+ and Cl^- concentrations were further increased in the aboveground tissues, and a clear positive concentration gradient of ions was observed from roots to stems to leaves (Fig. 12, lower panel). The active Na^+ exclusion early in the season changed into a strong building up of ions in the latter stages of development. It is worth to note that at this stage *gs* was strongly reduced, reaching values even below the detection level of the porometer. It is conceivable that at prolonged exposure to high salinity, the plants can no longer maintain low shoot Na^+ and Cl^- levels though ion exclusion from the roots, but accumulation in the leaves to extreme levels that are toxic even for quinoa are avoided by minimizing the transpiration stream that transports ions to the leaves.

It is noteworthy that [Cl^-] in young leaves was higher that [Na^+] in the shoots throughout the season. The high values for [Cl^-] reported in this study agree with reports in other halophytes. Cl^- accumulation has been considered as a compensatory mechanism to prevent charge imbalances (Flowers and Colmer, 2008). Given the high concentration of monovalent cations (Na^+ and K^+), Cl^- might be essential for electrical balance and the maintenance of a negative voltage (cytoplasm with respect to apoplast) (Teakle and Tyerman, 2010). Some of the consequences of Cl^- accumulation can be inhibition of gas exchange and reduction of nitrogen uptake and nitrate storage due to competitive transport of Cl^- and NO_3^- (Li et al., 2017). In our study, salt stressed plants showed lowered gas exchange measured by stomatal conductance. However, free nitrate content in photosynthetically active

leaves was strongly reduced only in selRiobamba (by 73 %) and mildly reduced in Pasto and Jessie (by 7 %), while Cl concentration in the three varieties was similar. This suggests that the competition of Cl^- and NO_3^- transport is not a general phenomenon in quinoa. Maintaining a high nitrate level in the leaves was not a determinant factor for the salt tolerance of these varieties. The lowest nitrate level under salt stress was found in selRiobamba, which showed the lowest seed yield reduction under severe salt stress.

When plants are transferred to a medium with high Na^+ (salt treatment), plant [K^+] typically decreases as [Na^+] rises (Flowers and Colmer, 2008). A major growth constraint of salt stress is a Na^+- induced K^+ deficiency that can disrupt cell metabolism. Our quinoa plants were able to maintain and even increase the levels of K^+ in the shoot also at the high [Na^+] after prolonged salt stress (10 weeks after start of the stress). Maintained or even elevated K^+ concentrations under high salinity is consistent with previous reports (Hariadi et al., 2011; Schmöckel et al., 2017; Shabala et al., 2013) and has been interpreted as evidence for the important role of K^+ in leaf osmotic adjustment under saline conditions (Shabala and Cuin, 2008). It may also protect the cells from metabolic failure due to a low K^+/Na^+ ratio. Mechanisms and transporters involved in the translocation of K^+ from root to shoot and its posterior distribution and cellular partitioning have been described in a number of studies (Ahmad and Maathuis, 2014; Benito et al., 2014; Szczerba et al., 2009). Under these stressful conditions, the ability of quinoa to retain a high concentration of K^+ in the cytosol is remarkable and may be essential for its salt tolerance, though it may come at a high metabolic cost.

Some authors have proposed that under salt stress epidermal bladder cells (EBCs) act as external storage organs for potentially toxic ions, and that therefore EBCs would play a pivotal role in the ion homeostasis of quinoa (Orsini et al., 2011; Zou et al., 2017). The low amount of ions accumulated in EBCs relative to the total amount in the leaves in our study do not suggest a strong contribution of EBCs to reducing the high levels of Na^+ and Cl^- in the leaves. EBCs constitute less than 1.3 % of the total fresh weight of young fully developed leaves. We think that the total EBCs volume is simply too low to hold enough salt to be considered salt storage organs under saline conditions. Further research is needed to understand the function of these specialized cells in quinoa.

4.2. Long term salt stress effects on growth: components of RGR and PSII

After the first three weeks of stress (400 mM NaCl) until the end of the crops cycle, stomatal conductance was reduced by more than 60 %. A similar reduction of the maximum CO_2-assimilation rate can be expected, which might lead to the photoinhibition of PSII and additional non-stomatal limitations to photosynthetic efficiency (Murata et al., 2007). In plants, salinity typically causes a rapid decline of PSII activity due to the inhibition of the repair of PSII caused by excessive ROS production (Murata et al., 2007). However, despite the severe stress applied in this study, the initial PSII light use efficiency of our quinoa plants (Fv/Fm ratio) was not decreased (Figure S3) which corroborates previous reports in quinoa (Shabala et al., 2013).

While PSII efficiency was not affected by salt, the relative growth was. The impact of salt stress on quinoa growth during the full crop cycle can be assessed using Relative Growth Rate analysis (RGR) because it factorises growth into physiological, morphological, anatomical and biochemical traits (Lambers and Poorter, 1992). The most important effect of salinity on quinoa RGR components was not on the relative investment in leaf growth (LWR), but on the morphology of the leaves (SLA); decreased SLA likely implies thicker leaves. The modified leaves have a decreased total area for transpiration and radiation interception, but increases photosynthetic capacity per surface area (as seen by an increased NAR). Therefore, both the initial light use efficiency (PSII) and the long term photosynthetic rate (NAR) were not affected by salt. A higher NAR associated with a lower SLA has been reported in several species (Montes Osorio et al., 2014). Gas exchange measurements reported after 4 weeks of the start of a salt treatment of 250 mM NaCl showed a lower maximum net CO_2-assimilation rate (Becker et al., 2017). In our long term assessment, we observed a higher NAR, which suggest a recovery of CO_2-assimilation after prolonged salt stress, even at lower stomatal conductance. This anatomical adaptation of quinoa leaves under severe stress (lower SLA with higher NAR) might explain that the RGR was reduced only by 10 %, a minor decrease compared to the impact of adverse conditions on the RGR of other species discussed in literature (Norris, 1982).

In conclusion, salt stress impacts the growth of quinoa directly, through a lowered metabolism (stomatal closure, less carbon assimilation, decrease in cell expansion) and indirectly, through several salt tolerance mechanisms examined in this study that come at a considerable metabolic cost (Tyerman et al., 2019).

4.3. Lessons from different stress levels: trade-off between survival and growth

The European varieties in our study displayed remarkable variation in growth and salinity responses. The salt treatments in the range of 100 − 300 mM NaCl might be considered mild stress for quinoa; even though seed yield was reduced, quinoa was still able to perform relatively well under these conditions compared to other grain crops. Under mild stress, varieties did not differ in their responses to salt. Under highly saline conditions (> 400 mM NaCl), seed yield was severely reduced in all the varieties, but Pasto was the most affected. This variety displayed a behaviour that deviated from the other varieties for several physiological traits. Pasto showed the highest reduction in SLA and transpiration, the lowest concentration Na^+ and Cl^- in young leaves, the highest concentration of K^+ in young leaves, and the lowest reduction in nitrate concentration in young leaves throughout the growing season. In addition, flowering, seed filling and seed setting times were delayed for Pasto (not shown). Its growth was more reduced than the other varieties, but the plants still appeared to be healthy, which is supported by the highest RWC, the highest NAR increase and an increase in chlorophyll content throughout the season. We speculate that Pasto employed a “survival” strategy with a more reduced growth rate, transpiration rate and higher rate of exclusion of Na^+. These adaptations allowed Pasto and Pasto-like varieties to survive longer, but at the trade-off of the very high reduction in growth rate and seed production.

Author statement

VJR performed the experiments, analyzed the data and wrote the original draft of the paper. LAT, CCG and NP performed the experiments and were part of the discussions of the outcomes of the research. RGFV helped to conceptualize the study, discussed the outcomes, reviewed and edited the manuscript. ENL and GVL coordinated and supervised this research, conceptualized the experimental design, contributed to the data analysis, guided the discussion of the outcomes, reviewed and edited the manuscript.

Funding

Viviana Jaramillo is financially supported by a scholarship from the Secretariat for Higher Education, Science, Technology and Innovation of the Republic of Ecuador (Contract Number 2015-AR2Q8878). This research was partially sponsored by the Graduate School Experimental Plant Sciences, Wageningen University, Wageningen, The Netherlands.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge the expert assistance of Geurt Versteeg and Andre Maassen in the greenhouse experiments.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2020.104146.

References

Adolf, V.I., Shabala, S., Andersen, M.N., Razzaghi, F., Jacobsen, S.E., 2012. Varietal differences of quinoa's tolerance to saline conditions. Plant Soil 357 (1), 117–129. https://doi.org/10.1007/s11104-012-1133-7.

Adolf, V.I., Jacobsen, S.-E., Shabala, S., 2013. Salt tolerance mechanisms in quinoa (Chenopodium quinoa Willd.). Environ. Exp. Bot. 92, 43–54. https://doi.org/10.1016/j.envexpbot.2012.07.004.

Ahmad, I., Maathuis, F.J.M., 2014. Cellular and tissue distribution of potassium: physiological relevance, mechanisms and regulation. J. Plant Physiol. 171 (9), 708–714. https://doi.org/10.1016/j.jplph.2013.10.016.

Ashraf, M., Khanum, A., 1997. Relationship Between Ion Accumulation and Growth in Two Spring Wheat Lines Differing in Salt Tolerance at Different Growth Stages. J. Agron. Crop. Sci. 178 (1), 39–51. https://doi.org/10.1111/j.1439-037X.1997.tb00349.x.

Becker, V.I., Goessling, J.W., Duarte, B., Caçador, I., Liu, F., Rosenqvist, E., Jacobsen, S.-E., 2017. Combined effects of soil salinity and high temperature on photosynthesis and growth of quinoa plants (*Chenopodium quinoa*). Funct. Plant Biol. 44 (7), 665–678. https://doi.org/10.1071/FP16370.

Benito, B., Haro, R., Amtmann, A., Cuin, T.A., Dreyer, I., 2014. The twins K+ and Na+ in plants. J. Plant Physiol. 171 (9), 723–731. https://doi.org/10.1016/j.jplph.2013.10.014.

Britto, D.T., Kronzucker, H.J., 2015. Sodium efflux in plant roots: What do we really know? J. Plant Physiol. 186–187, 1–12. https://doi.org/10.1016/j.jplph.2015.08.002.

Daliakopoulos, I.N., Tsanis, I.K., Koutroulis, A., Kourgialas, N.N., Varouchakis, A.E., Karatzas, G.P., Ritsema, C.J., 2016. The threat of soil salinity: a European scale review. Sci. Total Environ. 573, 727–739. https://doi.org/10.1016/j.scitotenv.2016.08.177.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179 (4), 945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x.

Geissler, N., Hussin, S., El-Far, M.M.M., Koyro, H.W., 2015. Elevated atmospheric CO2 concentration leads to different salt resistance mechanisms in a C3 (Chenopodium quinoa) and a C4 (Atriplex nummularia) halophyte. Environ. Exp. Bot. 118, 67–77. https://doi.org/10.1016/j.envexpbot.2015.06.003.

Gonzalez, J.A., Eisa, S.S.S., Hussin, S.A.E.S., Prado, F.E., 2015. Quinoa: An Incan crop to face global changes in agriculture. Quinoa: Improvement and Sustainable Production. doi:10.1002/9781118628041.ch1 In press.

Hariadi, Y., Marandon, K., Tian, Y., Jacobsen, S.E., Shabala, S., 2011. Ionic and osmotic relations in quinoa (Chenopodium quinoa Willd.) plants grown at various salinity levels. J. Exp. Bot. 62 (1), 185–193. https://doi.org/10.1093/jxb/erq257.

Hinojosa, L., González, J.A., Barrios-Masias, F.H., Fuentes, F., Murphy, K.M., 2018. Quinoa abiotic stress responses: a review. Plants Basel (Basel) 7 (4), 106. https://doi.org/10.3390/plants7040106.

Hirich, A., Jelloul, A., Choukr-Allah, R., Jacobsen, S.-E., 2014. Saline water irrigation of quinoa and chickpea: seedling rate, stomatal conductance and yield responses. J. Agron. Crop. Sci. 200 (5), 378–389. https://doi.org/10.1111/jac.12072.

Jacobsen, S.E., Mujica, A., Jensen, C.R., 2003. The resistance of quinoa (Chenopodium quinoaWilld.) to adverse abiotic factors. Food Rev. Int. 19 (1–2), 99–109. https://doi.org/10.1081/fri-120018872.

Jarvis, D.E., Ho, Y.S., Lightfoot, D.J., Schmöckel, S.M., Li, B., Borm, T.J.A., Ohyanagi, H., Mineta, K., Michell, C.T., Saber, N., Kharbatia, N.M., Rupper, R.R., Sharp, A.R., Dally, N., Boughton, B.A., Woo, Y.H., Gao, G., Schijlen, E.G.W.M., Guo, X., Momin, A.A., Negrão, S., Al-Babili, S., Gehring, C., Roessner, U., Jung, C., Murphy, K., Arold, S.T., Gojobori, T., Linden, C.G.V.D., Van Loo, E.N., Jellen, E.N., Maughan, P.J., Tester, M., 2017. The genome of Chenopodium quinoa. Nature 542 (7641), 307–312. https://doi.org/10.1038/nature21370.

Julkowska, M.M., Testerink, C., 2015. Tuning plant signaling and growth to survive salt. Trends Plant Sci. 20 (9), 586–594. https://doi.org/10.1016/j.tplants.2015.06.008.

Lambers, H., Poorter, H., 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv. Ecol. Res. 23. https://doi.org/10.1016/s0065-2504(08)60148-8.

Lebaudy, A., Véry, A.-A., Sentenac, H., 2007. K+ channel activity in plants: genes, regulations and functions. FEBS Lett. 581 (12), 2357–2366. https://doi.org/10.1016/j.febslet.2007.03.058.

Li, B., Tester, M., Gilliham, M., 2017. Chloride on the move. Trends Plant Sci. 22 (3), 236–248. https://doi.org/10.1016/j.tplants.2016.12.004.

Maathuis, F.J.M., Ahmad, I., Patishtan, J., 2014. Regulation of Na+ fluxes in plants. Front. Plant Sci. 5 (467). https://doi.org/10.3389/fpls.2014.00467.

Mastebroek, H.D., Van Loo, E.N., Dolstra, O., 2002. Combining ability for seed yield traits of Chenopodium quinoa breeding lines. Euphytica 125 (3), 427–432. https://doi.org/10.1023/a:1016030129541.

Maughan, P.J., Turner, T.B., Coleman, C.E., Elzinga, D.B., Jellen, E.N., Morales, J.A., Udall, J.A., Fairbanks, D.J., Bonifacio, A., 2009. Characterization of Salt overly Sensitive 1 (SOS1) gene homoeologs in quinoa (Chenopodium quinoa Willd.). Genome 52 (7), 647–657. https://doi.org/10.1139/g09-041.

Mishra, A., Tanna, B., 2017. Halophytes: potential resources for salt stress tolerance genes and promoters. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00829.

Møller, I.S., Tester, M., 2007. Salinity tolerance of Arabidopsis: a good model for cereals? Trends Plant Sci. 12 (12), 534–540. https://doi.org/10.1016/j.tplants.2007.09.009.

Montes Osorio, L.R., Torres Salvador, A.F., Jongschaap, R.E.E., Azurdia Perez, C.A., Berduo Sandoval, J.E., Trindade, L.M., Visser, R.G.F., van Loo, E.N., 2014. High level of molecular and phenotypic biodiversity in Jatropha curcas from Central America compared to Africa, Asia and South America. BMC Plant Biol. 14 (1). https://doi.org/10.1186/1471-2229-14-77.

Munns, R., James, R.A., Gilliham, M., Flowers, T.J., Colmer, T.D., 2016. Tissue tolerance: an essential but elusive trait for salt-tolerant crops. Funct. Plant Biol. 43 (12), 1103–1113.

Munns, R., Passioura, J.B., Colmer, T.D., Byrt, C.S., 2020. Osmotic adjustment and energy limitations to plant growth in saline soil. New Phytol. 225 (3), 1091–1096. https://doi.org/10.1111/nph.15862.

Murata, N., Takahashi, S., Nishiyama, Y., Allakhverdiev, S.I., 2007. Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta - Bioenergetics 1767 (6), 414–421. https://doi.org/10.1016/j.bbabio.2006.11.019.

Negrão, S., Schmöckel, S.M., Tester, M., 2017. Evaluating physiological responses of plants to salinity stress. Ann. Bot. 119 (1), 1–11. https://doi.org/10.1093/aob/mcw191.

Norris, I.B., 1982. Soil moisture and growth of contrasting varieties of *Lolium*, *Dactylis* and *Festuca* species. Grass Forage Sci. 37 (4), 273–283. https://doi.org/10.1111/j.1365-2494.1982.tb01607.x.

Orsini, F., Accorsi, M., Gianquinto, G., Dinelli, G., Antognoni, F., Carrasco, K.B.R., Martinez, E.A., Alnayef, M., Marotti, I., Bosi, S., Biondi, S., 2011. Beyond the ionic and osmotic response to salinity in Chenopodium quinoa: functional elements of successful halophytism. Funct. Plant Biol. 38 (10), 818–831. https://doi.org/10.1071/fp11088.

Razzaghi, F., Jacobsen, S.-E., Jensen, C.R., Andersen, M.N., 2015. Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought – mechanisms of tolerance. Funct. Plant Biol. 42 (2), 136–148. https://doi.org/10.1071/FP14132.

Roy, S.J., Tucker, E.J., Tester, M., 2011. Genetic analysis of abiotic stress tolerance in crops. Curr. Opin. Plant Biol. 14 (3), 232–239. https://doi.org/10.1016/j.pbi.2011.03.002.

Rubio, F., Nieves-Cordones, M., Horie, T., Shabala, S., 2020. Doing 'business as usual' comes with a cost: evaluating energy cost of maintaining plant intracellular K+ homeostasis under saline conditions. New Phytol. 225 (3), 1097–1104. https://doi.org/10.1111/nph.15852.

Ruiz-Carrasco, K., Antognoni, F., Coulibaly, A.K., Lizardi, S., Covarrubias, A., Martínez, E.A., Molina-Montenegro, M.A., Biondi, S., Zurita-Silva, A., 2011. Variation in salinity tolerance of four lowland genotypes of quinoa (Chenopodium quinoa Willd.) as assessed by growth, physiological traits, and sodium transporter gene expression. Plant Physiol. Biochem. 49 (11), 1333–1341. https://doi.org/10.1016/j.plaphy.2011.08.005.

Sairam, R.K., Rao, K.V., Srivastava, G.C., 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 163 (5), 1037–1046. https://doi.org/10.1016/S0168-9452(02)00278-9.

Schmöckel, S.M., Lightfoot, D.J., Razali, R., Tester, M., Jarvis, D.E., 2017. Identification of putative transmembrane proteins involved in salinity tolerance in chenopodium quinoa by integrating physiological data, RNAseq, and SNP analyses. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.01023.

Shabala, S., 2013. Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. Ann. Bot. 112 (7), 1209–1221. https://doi.org/10.1093/aob/mct205.

Shabala, S., Cuin, T.A., 2008. Potassium transport and plant salt tolerance. Physiol. Plant. 133 (4), 651–669. https://doi.org/10.1111/j.1399-3054.2007.01008.x.

Shabala, L., Mackay, A., Tian, Y., Jacobsen, S.-E., Zhou, D., Shabala, S., 2012. Oxidative stress protection and stomatal patterning as components of salinity tolerance mechanism in quinoa (Chenopodium quinoa). Physiol. Plant. 146 (1), 26–38. https://doi.org/10.1111/j.1399-3054.2012.01599.x.

Shabala, S., Hariadi, Y., Jacobsen, S.E., 2013. Genotypic difference in salinity tolerance in quinoa is determined by differential control of xylem Na+ loading and stomatal density. J. Plant Physiol. 170 (10), 906–914. https://doi.org/10.1016/j.jplph.2013.01.014.

Szczerba, M.W., Britto, D.T., Kronzucker, H.J., 2009. K+ transport in plants: physiology and molecular biology. J. Plant Physiol. 166 (5), 447–466. https://doi.org/10.1016/j.jplph.2008.12.009.

Teakle, N.L., Tyerman, S.D., 2010. Mechanisms of Cl- transport contributing to salt tolerance. Plant Cell Environ. 33 (4), 566–589. https://doi.org/10.1111/j.1365-3040.2009.02060.x.

Tyerman, S.D., Munns, R., Fricke, W., Arsova, B., Barkla, B.J., Bose, J., Bramley, H., Byrt, C., Chen, Z., Colmer, T.D., Cuin, T., Day, D.A., Foster, K., Gilliham, M., Henderson, S.W., Horie, T., Jenkins, C.L.D., Kaiser, B.N., Katsuhara, M., Plett, D., Miklavcic, S.J., Roy, S.J., Rubio, F., Shabala, S., Shelden, M., Soole, K., Taylor, N.L., Tester, M., Watt, M., Wege, S., Wegner, L.H., Wen, Z., 2019. Energy costs of salinity tolerance in crop plants. New Phytol. 221 (1), 25–29. https://doi.org/10.1111/nph.15555.

Véry, A.-A., Nieves-Cordones, M., Daly, M., Khan, I., Fizames, C., Sentenac, H., 2014. Molecular biology of K+ transport across the plant cell membrane: What do we learn from comparison between plant species? J. Plant Physiol. 171 (9), 748–769. https://

doi.org/10.1016/j.jplph.2014.01.011.
Zou, C., Chen, A., Xiao, L., Muller, H.M., Ache, P., Haberer, G., Zhang, M., Jia, W., Deng, P., Huang, R., Lang, D., Li, F., Zhan, D., Wu, X., Zhang, H., Bohm, J., Liu, R., Shabala, S., Hedrich, R., Zhu, J.-K., Zhang, H., 2017. A high-quality genome assembly of quinoa provides insights into the molecular basis of salt bladder-based salinity tolerance and the exceptional nutritional value. Cell Res. 27 (11), 1327–1340. https://doi.org/10.1038/cr.2017.124.
Zurita-Silva, A., Fuentes, F., Zamora, P., Jacobsen, S.E., Schwember, A.R., 2014. Breeding quinoa (Chenopodium quinoa Willd.): potential and perspectives. Mol. Breed. 34 (1), 13–30. https://doi.org/10.1007/s11032-014-0023-5.