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Research article

Salares versus coastal ecotypes of quinoa: Salinity responses in Chilean landraces from contrasting habitats

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is a highly salt-tolerant species subdivided into five ecotypes and exhibiting broad intra-specific differences in tolerance levels. In a greenhouse study, Chilean landraces belonging either to the *salares* (R49) or coastal lowlands (VI-1, Villarrica) ecotype with contrasting agro-ecological origins were investigated for their responses to high salinity. The effects of two levels of salinity, 100 (T1) and 300 (T2) mM NaCl, on plant growth and on some physiological parameters were measured. Leaf and root Na^+ accumulation differed among landraces. T2 reduced growth and seed yield in all landraces with maximum inhibition relative to controls in R49. Salinity negatively affected chlorophyll and total polyphenol content (TPC) in VI-1 and Villarrica but not R49. Germination on saline or control media of seeds harvested from plants treated or not with NaCl was sometimes different; the best performing landrace was R49 insofar as 45–65% of seeds germinated on 500 mM NaCl-containing medium. In all landraces, average seedling root length declined strongly with increasing NaCl concentration, but roots of R49 were significantly longer than those of VI-1 and Villarrica up to 300 mM NaCl. Salt caused increases in seed TPC relative to controls, but radical scavenging capacity was higher only in seeds from T2 plants of R49. Total SDS-extractable seed proteins were resolved into distinct bands (10–70 kDa) with some evident differences between landraces. Salt-induced changes in protein patterns were landrace-specific. The responses to salinity of the *salares* landrace are discussed in relation to its better adaptation to an extreme environment.

1. Introduction

Adaptation of agriculture to changing climatic conditions, expected to entail increasing soil salinization, relies on the use of suitable crops displaying resistance to abiotic and biotic stresses. Researches on responses and tolerance to salt, as well as the definition of parameters for screening and selection, have mainly focused on conventional crops most of which are glycophytes. A few investigations on screening of halophytic species and their responses to saline conditions is starting to draw the attention of researchers in view of promoting “halophyte agriculture” (Rozema and Schat, 2013).

Basic research on morphological and physiological salt stress-induced responses and mechanisms of adaptation in both glycophytes and halophytes is of paramount importance for plant breeding and selection of most suitable genotypes (Shabala et al. 2013). Studies on plant salt tolerance during different ontogenetic phases is also important for determining saline limits and for stabilizing crop performance under saline conditions, since tolerance might vary at each phase (Munns and Tester, 2008).

Quinoa (*Chenopodium quinoa* Willd.), an ancient Andean seed-producing crop, belongs to the Amaranthaceae, a family that comprises many halophyte species. In the last decades, quinoa has attracted the attention of researchers and consumers worldwide because of its tolerance to unfavorable environmental conditions such as salinity, drought, and frost (Bosque-Sánchez et al. 2003; Jacobsen et al. 2012; Razzaghi et al. 2011; Adolf et al. 2013; Ruiz et al. 2015) and its highly nutritious seeds (Vega-Gálvez et al.

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2010). Its adaptability derives from the fact that the species is cultivated since about 7000 years around Lake Titicaca in the Andean highlands (*altiplano*) from where it spread as far north as Ecuador and down to southern Chile, and from 3800 m above sea level to coastal lowlands areas. This broad diversification in terms of native habitats accompanied by a great genetic diversity has led to the identification of five ecotypes (or landraces adapted to different environments): *salares* (salt flats), highlands, inter-Andean valleys, *yungas*, and coastal lowlands (Tapia, 2015). The *salares* of the Andes are found principally in southern Bolivia, northern Chile and Argentina. These highland deserts are extremely arid (<200 mm precipitation/year) and temperatures often fall below −20 °C. Quinoa is the only crop that can grow under these edapho-climatic conditions (Fuentes et al. 2009). In central and southern Chile, quinoa can grow at sea level; here annual rainfall, distributed throughout the year, ranges from 400 to 1500–2000 mm and soils have a high water retention capacity. Consequently, quinoa exhibits a broad intra-specific range of tolerance to saline conditions, as revealed by comparative studies on many different accessions, landraces, and cultivars (Gómez-Pando et al. 2010; Adolf et al. 2012; Ruiz et al. 2015; Peterson and Murphy, 2015). Thus, even in a halophytic crop such as quinoa, high salinity can negatively influence growth as well as seed yield (Koyro and Eisa, 2008; Hariadi et al. 2011; Orsini et al. 2011). However, comparative studies have shown that the extent to which these parameters are affected by salinity is strongly genotype-dependent (Adolf et al. 2012; Gómez-Pando et al. 2010). Genotypes originating from salinity-affected areas are generally better adapted to salt stress than those from non-saline (or less arid) areas. This variability represents an important resource for selection and breeding that opens the way for even higher tolerance in cultivars adapted to different altitudes, latitudes, soil, and climatic conditions. In terms of basic research, comparing responses in different genotypes with variable sensitivities to salt stress is a useful approach towards gaining knowledge on the morphological, physiological, and molecular mechanisms responsible for salt tolerance in quinoa and in other halophytes (Ruiz et al. 2015).

In Chile, all landraces and accessions belong either to the *salares* or to the coastal lowlands ecotype; they form genetically distinct groups with evident differences in terms of geographic origin, soil/climate conditions, and traditional cultural practices with different levels of adaptation to altitude, drought, salinity and day length (Bazile et al. 2015). Delatorre-Herrera and Pinto (2009) go so far as to suggest that the quinoa landraces from the northern *altiplano* are closer to halophytes or facultative halophytes, while the selections from the south could be closer to glycophytes. In a comparative study by Adolf et al. (2012), Chilean coastal lowlands landraces were among those with the lowest salinity tolerance in terms of biomass and height. Chilean landraces belonging to these two ecotypes have not been fully characterized from an eco-physiological standpoint except in a few cases. In a comparative study between two accessions from the northern highland region and two from the less salinity-prone southern region, Delatorre-Herrera and Pinto (2009) reported a higher germination rate under 400 mM NaCl of seeds from the highlands and showed that the osmotic and ionic components of salt stress varied between landraces. Four Chilean landraces originating along a north-south gradient were also compared by Ruiz-Carrasco et al. (2011). Germination rate and seedling growth on NaCl-supplemented media, as well as several physiological and molecular parameters, such as proline and polyamine contents, and transcript levels of ion homeostasis-related genes (*CqSOS1* and *CqNHX*) measured at the seedling stage, distinguished the southernmost landrace from the others.

In the present work, we assessed how a Chilean landrace of quinoa from the northern hyperarid *altiplano* belonging to the *salares* ecotype (R49) and two belonging to the coastal lowlands ecotype but with origins in different habitats: the central zone with a semi-arid/Mediterranean climate (*secano costero*), and the southern humid zone (Pizarro et al. 2012), responded to salinity at the end of their growth cycle. We attempted to answer the following questions: a) is the level of tolerance determined by original habitat, b) which parameters are indicators of stress tolerance, c) is seed quality affected by salinity? Plants were grown in pots under greenhouse conditions and irrigated with saline solutions (100 and 300 mM NaCl) from seedling establishment up to harvest. In order to evaluate their long-term adaptation, plant growth and physiological parameters were determined during and at the end of the growth cycle. Productivity in terms of seed production was evaluated at harvest. Germinability and seedling growth on saline media (*in vitro* test) of seeds from salt-grown and control plants were compared in the different landraces. Finally, seed quality was evaluated in terms of total protein concentration and profile, total polyphenol content, and antioxidant activity.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of three Chilean landraces of *C. quinoa* (Willd.), one belonging to the *salares* ecotype (R49) and two, VI-1 and Villarrica (VR), to the coastal lowlands ecotype were collected along an altitudinal gradient from the arid northern highland with saline soils (3800 m a.s.l.) to sea level, and along a latitudinal gradient of *ca.* 2500 km down to the rainier central/southern region (*ca.* 34° to 39°S) with higher precipitation and non-saline soils (Table 1). All seeds were obtained from the National Seed Bank of Chile managed by INIA-Intihuasi (Vicuña, Chile).

Greenhouse experiments were carried out with vernalized seeds sown in 20-L plastic pots containing a garden soil:sand (1:1) mixture. When plants had four to six well-expanded leaves (*ca.* 34 days after sowing) salt treatment was started by irrigating pots weekly with 0, 100 or 300 mM NaCl solutions (control, T1 and T2, respectively); all the pots (control and salt-treated) were also watered weekly with 100–200 mL water supplemented with Phostrogen (N:P:K 10:10:27; 0.4 g L^{-1}; Bayer Garden, Cambridge, UK). Plants were grown (October to April) under natural daylight conditions and the temperature was maintained at *ca.* 23 ± 3 °C.

Phenology was monitored weekly starting 36 days after sowing up to the end of the experiment (i.e., at harvest). Plant height measurement and leaf sampling were carried out 84 days after sowing when all control plants had reached the milky grain stage. Samples (n = 6) from fully-expanded mature leaves were harvested, immediately frozen in liquid N_2 and stored at −80 °C until use for pigment, polyphenol and flavonoid determinations. Whole plants (leaves, stems, roots and seeds) were harvested starting from 110 days after the first salt treatment, depending on the landrace. Whole plant, root and leaf dry weight (DW) was evaluated by drying in an oven at 40 °C for five days until constant weight. Dried samples were used to measure Na^+ concentration in plant organs. Seeds were collected, weighed and stored in an air-tight container at 4 °C until use; aliquots of the seeds were freeze-dried.

2.2. Soil electrical conductivity and plant sodium content determinations

The electrical conductivity (EC) in the soil was measured, at the end of the experiment, by taking three replicates of 150 g of soil from each pot. The samples were water saturated and after 24 h the saturated samples were filtered under vacuum. EC was determined

Table 1
The Chilean landraces studied, ecotypes, and geographical origins.

Landrace	Ecotype	Provenance	Lat. (W); long. (S)	Altitude (m.a.s.l.)	Martonne Index[a]
R49	*salares*	Colchane county	19°25′; 68°35′	3800	0.22
VI-1	coastal lowlands	Pichilemu	34°28′; 72°00′	0	284
VR	coastal lowlands	Villarrica	39°16′; 72°13′	227	1072

[a] The Martonne index considers precipitation, temperature and soil texture; the closer the index is to zero, the higher the potential osmotic/salt stress.

using a bench OakTon conductivity meter (model CON510 series, Vernon Hills, Illinois, USA). At harvest, [Na^+] in leaves, stems, and roots (*ca*. 500 mg DW) was quantified as described by Tapia et al. (2013). Each replicate was digested with 10 mL of Milli-Q H_2O, 3 mL of HNO_3 and 2 mL of H_2O_2 in an autoclave. The [Na^+] in the digested extracts was determined by Atomic Absorption Spectrometry (AAS).

2.3. Photosynthetic pigments

Pigment extraction (chlorophylls and total carotenoids) was carried out in dim light to minimize photo-transformation of chlorophyll. Leaf samples (equivalent to 2 cm^2; *ca* 50 mg FW) were ground in a chilled mortar with 10 volumes of 100% (v/v) cold methanol. The extract was centrifuged at 5000 rpm for 10 min at 4 °C, and the supernatant was kept at 4 °C. The pellet was re-suspended in the same solution and centrifuged three times at 5000 rpm for 10 min at 4 °C. The supernatants were pooled and the absorbance determined spectrophotometrically in a microplate spectrophotometer (Epoch, BioTek instruments; Vermont, USA). Readings were made at 665 for Chl*a*, 652 for Chl*b*, and 470 nm for total carotenoids (the sum of carotenoids and xanthophylls, c + x). Pigment concentrations were estimated based on the specific absorbance coefficients in methanolic extracts as previously described by Lichtenthaler (1987). Results are expressed as mg g^{-1} FW.

2.4. Preparation of methanol extracts

2.4.1. Leaves

About 50 mg of fresh leaf tissue were ground in 10 vols of 100% (v/v) methanol. The homogenate was sonicated for 30 min and centrifuged for 20 min at 4000 rpm. The supernatant was separated and the pellet washed twice with 100% methanol and centrifuged 5 min at 3000 rpm. The supernatants were pooled and stored at −20 °C until use.

2.4.2. Seeds

For polyphenol determination and antioxidant activity assays, 20 freeze-dried seeds (*ca*. 50 mg DW) were ground to a powder in a mortar. After adding 2 mL 100% methanol, the suspension was sonicated for 30 min and then centrifuged for 20 min at 10,000 rpm. After collecting the supernatant, the pellet was re-suspended with 1 mL methanol and left for 40 min at 30 °C; after centrifuging and separating the supernatant, the pellet was re-extracted by adding 1 mL methanol and leaving overnight in agitation (200 rpm) at RT. The supernatants were pooled and taken to dryness under reduced pressure. The dry extract was re-suspended in 500 μL methanol and stored at −20 °C until use. Seed methanol extracts were performed on three biological replicates.

2.5. Determination of total phenolics content

Total phenolics content (TPC) in leaves and seeds was determined using the Folin-Ciocalteu (FC) reagent according to Singleton and Rossi (1965) with some modifications; 50 μL of the methanolic extract were mixed with 0.25 mL of FC reagent (previously diluted 10-fold with distilled water) and 0.5 mL distilled water were added. The homogenate was incubated for 1 min at room temperature and then 0.8 mL of 20% (w/v) Na_2CO_3 was added. After incubation at 40 °C for 30 min, the absorbance was measured spectrophotometrically at 760 nm. The TPC was evaluated from a gallic acid standard curve and is expressed as mg gallic acid equivalents (GAE) g^{-1} FW (leaves) or DW (seeds). The total flavonoid content was determined with $AlCl_3$ according to Zhishen et al. (1999) using rutin as standard. The leaf methanolic extract (50 μL) was added to 0.45 mL of 100% (v/v) methanol followed by 0.5 mL of 2% (w/v) $AlCl_3$ in methanol. This reaction mixture was incubated for 15 min at room temperature. Finally, the absorbance of 0.25 mL of reaction mixture was measured spectrophotometrically at 430 nm. Data are expressed as mg rutin equivalents (RE) g^{-1} FW.

2.6. Seed production and germination

Seed production in all three landraces was evaluated by weighing the seeds produced by each plant at the end of the experiment (i.e., at harvest); the FW and DW of 20 seeds was also determined. To assess the germinability of seeds harvested from plants grown under saline or non-saline conditions an *in-vitro* test was performed. Seeds were sterilized with 70% (v/v) ethanol followed by 10% commercial bleach, and then rinsed several times in sterile water. After 48 h of vernalization at 4 °C, seeds were sown in agar plates containing half-strength Murashige and Skoog (1962) medium added with 0, 100, 300 or 500 mM NaCl. Plates were placed vertically in a growth chamber at 24 °C with an 8/16 h dark/light photoperiod and a light intensity of 120 μE m^{-2} s^{-1}. Germination (%) was evaluated 10 days after sowing, when seedling root and hypocotyl length was evaluated by ImageJ software (Abramoff et al. 2004).

2.7. Antioxidant assays

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) and FRAP-Ferrozine (FZ) tests, the latter performed in order to evaluate the reducing potential of extracts towards the redox couple Fe^{3+}/Fe^{2+}, were performed according to Venditti et al. (2013). In the assays, the methanolic extract was tested at several concentrations (by diluting it 1:1, 1:2, 1:4, 1:8) and Trolox was used as standard in a concentration range of 0–33 μM. The antioxidant capacity was expressed as Total Antioxidant Capacity (TAC) expressed as μM Trolox equivalent mg^{-1} DW.

2.8. Seed protein analyses

2.8.1. Total extractable protein content

Freeze-dried seeds (50 mg DW) were ground to a powder. The flour was defatted overnight with hexane under continuous stirring and then air-dried at room temperature. Total proteins were extracted under reducing conditions in the following buffer solution: 4% sodium dodecyl sulfate (SDS), 20% glycerol, 200 mM 2-mercaptoethanol in 100 mM Tris–HCl, pH 6.8. Buffer was added

(0.01 mL mg^{-1} DW) and the suspension mechanically homogenized then incubated for 1 h at 22 °C and finally centrifuged for 10 min at 10,000 rpm. Protein concentration was determined spectrophotometrically at 562 nm using the bicinchoninic acid kit (Sigma–Aldrich, Milano, Italy) and bovine serum albumin (BSA) as standard (Chan and Wasserman, 1993).

2.8.2. Electrophoresis and gel staining

Quinoa seeds were washed many times with cold water to remove saponins until there was no more foam in the wash water, and then dried at 50 °C until 15 ± 3% moisture. They were then ground with a mortar and pestle and the flour defatted as described above, air-dried at room temperature, and finally stored at 4 °C until use. Total proteins were extracted and measured as described above.

All electrophoretic runs were performed with the Mini Protean III apparatus (Bio-Rad Laboratories, Segrate, Italy). Proteins (40 μg $lane^{-1}$) were separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli et al. (1970) using a 12 or 15% acrylamide separating gel with a 4% acrylamide stacking gel. The molecular mass standard was the Biomol (Hamburg, Germany) BLUEplus prestained Protein Ladder (10–180 kDa). Samples were prepared in buffer containing 2% SDS, 10% glycerol, 200 mM 2-mercaptoethanol and 0.01 mg/mL bromophenol blue in 63 mM Tris–HCl (pH 6.8) and then boiled for 5 min prior to loading. Gels were fixed at room temperature in methanol/glacial acetic acid/water (50/5/45, v/v) for 20 min, then in 50% methanol for 10 min. After fixing, gels were washed twice in deionized water (10 min each) and stained with silver staining as described by Shevchenko et al. (1996) with minor modifications. Briefly, gels were incubated for 1 min at RT in 0.02% sodium thiosulphate and washed twice with deionized water for 5 min. Gels were then incubated in 0.1% silver nitrate solution for 20 min at 4 °C and then washed twice as described above. Staining was developed with 0.04% formaldehyde in 2% sodium carbonate. The process was stopped by adding 5% acetic acid. Protein profiles and densitometry were analyzed using the ImageJ software.

2.9. Statistical analysis

Two independent experiments were performed. Each experiment consisted of three pots per treatment (0, 100 and 300 mM NaCl), each containing one plant per landrace, and set up according to a randomized block design. Statistical analyses were carried out for all dependent variables measured in the experiment. Significant differences between controls and treatment within a landrace were tested by Tukey's *post-hoc* test for multiple comparisons when the one-way analysis of variance (ANOVA) indicated significant differences ($P < 0.05$). Data on germination percentages were compared by the χ^2 test. To determine the overall significances between groups, a two-way factorial analysis of variance (two-way ANOVA) was used with salt treatment and landrace as factors. Mean comparisons were made by applying the DCG *post-hoc* test using InfoStat software (Di Rienzo et al. 2014). Differences were considered significant at $P < 0.05$.

3. Results

3.1. Soil conductivity and plant Na^+ accumulation

Electrical conductivity (EC) was measured at the end of the experiment in salt-irrigated and control soil. With the lower concentration (100 mM NaCl, T1) average EC (32.2 dS m^{-1}) increased by four-fold above control levels; with 300 mM NaCl (T2), soil average EC reached up to six-fold that of controls (51.4 dS m^{-1}; data not shown).

Leaves of control plants accumulated from a minimum of *ca*. 3.0 (R49) to a maximum of 7.2 (VR) mg Na^+ g^{-1} DW (Fig. 1A). Under T1, leaves of VI-1 and VR accumulated about twice more Na^+ than those of R49. Under T2, leaves accumulated more Na^+ than under T1; in VI-1 it was about two-fold higher, reaching the highest leaf [Na^+] (163.9 mg g^{-1} DW), while the lowest (<80 mg g^{-1} DW) was found in R49. In general, the stem of salt-treated plants accumulated less Na^+ than leaves and roots at both concentrations of NaCl (data not shown). On average, roots of control plants accumulated 2.6 ± 0.9 mg g^{-1} DW Na^+ and, under T1, from *ca*. 28–35 mg g^{-1} DW Na^+ (Fig. 1B). Under T2, roots accumulated up to twice or more the amount of Na^+ found in T1 plants; R49, VI-1 and VR accumulated similar amounts of the ion.

The leaf-to-root [Na^+] ratio ranged from 1.2 to 3.1 under T1 and declined to 0.9, 2.5 and 1.5 in R49, VI-1 and VR, respectively under T2 (data not shown). The ratio never increased in T2 plants versus T1 and thus, R49 was the only landrace to exhibit a ratio <1.0 after this salt treatment (T2).

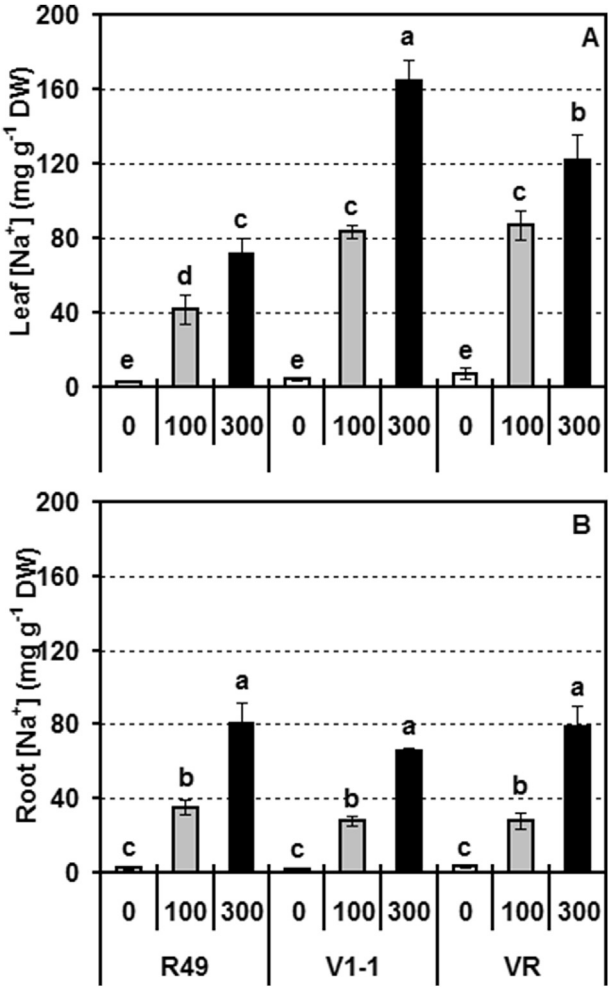

Fig. 1. Sodium concentrations at the end of the experiment (110 days after the onset of salt treatment) in leaves (A) and roots (B) of three Chilean landraces of C. *quinoa* irrigated with 0, 100 and 300 mM NaCl. Data are means (±SE) of six independent leaf samples (three biological replicates). Letters above the bars indicate significant differences ($P < 0.05$) by two-way ANOVA (genotype x treatment).

3.2. Plant phenology and growth

The landraces from central/southern Chile are considered early-to mid-ripening, while R49 is a late-ripening landrace. Harvest time was not substantially affected by salt in any of the three landraces, even though some of the phenological phases were delayed relative to their respective controls (Supplementary Material, Figure S1). In R49, the vegetative and reproductive phases were longer than in the other landraces under control conditions; in salt-treated plants, the inflorescence/flowering stages were delayed and longer than in controls, and the grain development/filling phases were shorter relative to controls and compared to the other landraces. Yellowing leaves became visible earlier in salt-treated plants than in control ones of R49 and VI-1 (data not shown).

Untreated plants of R49 were the tallest (Figs. 2A and 3A) and, at the end of the experiment, had the longest roots (Fig. 2B); there were no significant differences in stem height between the other two landraces (Fig. 3A). While T1 did not reduce plant height except in R49, T2 significantly reduced it in all landraces; maximum growth inhibition relative to controls was observed in R49 (about 50%), while it was only about 30–40% in the other landraces.

Landraces R49 and VR had higher total plant dry biomass than VI-1 under non-saline conditions (Fig. 3B). Total dry biomass was not affected by T1 relative to controls in VI-1 and VR whereas R49 was negatively affected (about 25% inhibition). Plant DW of VI-1 was unaffected by T2, while the others landraces had a significantly reduced DW, especially R49 (about 60% relative to controls). In control plants, leaf DW differed significantly between R49 and central-southern landraces (VI-1 and VR, respectively). Leaf DW under salinity increased in VR relative to controls, without differences between T1 and T2, while in R49 leaf DW declined significantly under T2 (Fig. 3C). In control and T1 plants, root DW per plant also differed significantly among landraces; T1 had a strongly inhibitory effect only in R49, while T2 always negatively affected root biomass (up to 74–87% inhibition in R49; Fig. 3D).

Internode lengths were also measured (and grouped into classes) and the frequency (number of internodes plant^{-1} for each class) established for all three landraces. Both T1 and, especially T2, enhanced the frequency of shorter internodes (Supplementary material, Figure S2).

3.3. Chlorophyll and carotenoid contents

Control plants of R49 had the lowest Chl contents (Table 2). Chl*a* concentration significantly decreased relative to untreated controls under T1 only in VI-1 and VR; under T2, both VI-1 and VR, but not R49, had lower Chl*a* contents; Chl*b* content was not affected by salinity. Both concentrations of salt caused significant decreases in carotenoid concentrations in R49 and VI-1 (25% and 50%, respectively) but not in VR (Table 2).

3.4. Leaf total polyphenol and flavonoid contents

In control plants, total phenolics content (TPC) at the end of the growth cycle was lower in R49 (ca. 8 mg g^{-1} FW) than in VI-1 and VR (Table 2). While salt-grown plants (both T1 and T2) of R49 exhibited higher TPC than controls, the other two landraces contained significantly less (up to 60%) polyphenols than controls. The pattern of total flavonoid accumulation in control plants reflected that of polyphenols being higher in VI-1 and VR and lower in R49.

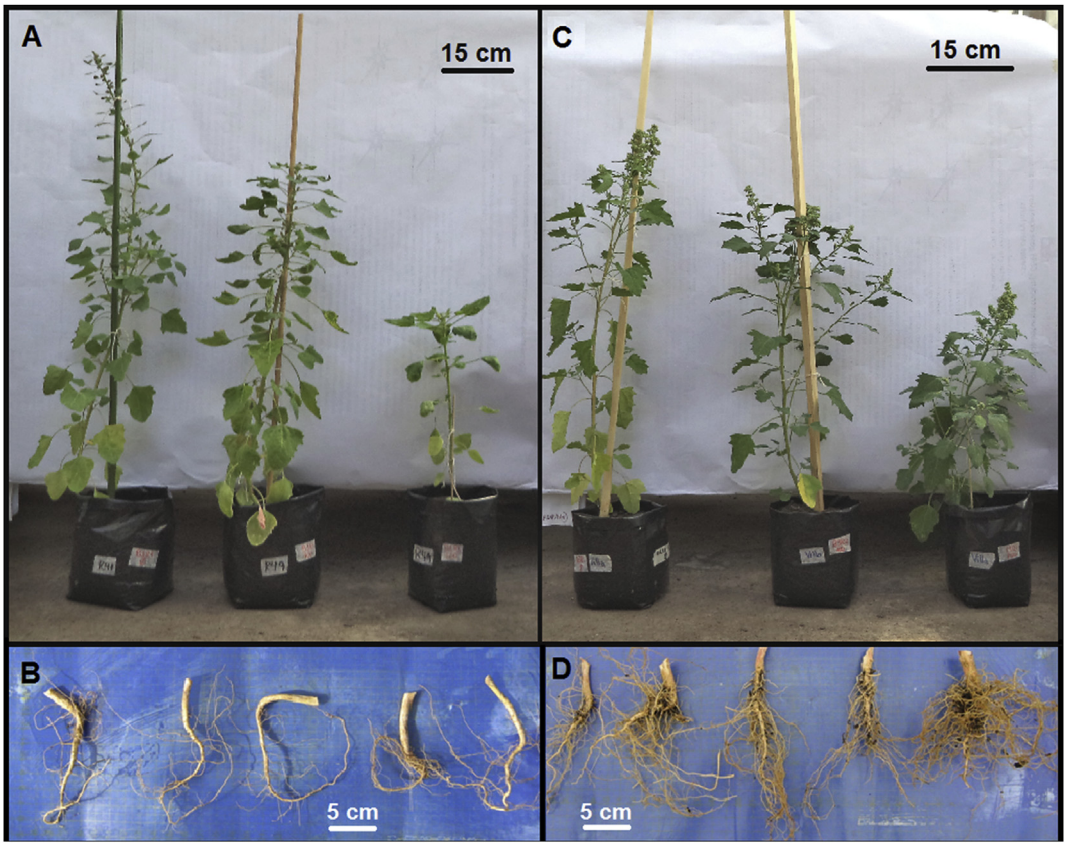

Fig. 2. Quinoa shoots of the *salares* (A) and of a coastal lowlands (C) landrace grown with 0 (left), 100 (middle) and 300 (right) mM NaCl, 45 days after the start of salt treatment; roots of untreated plants of the *salares* (B) and of a coastal lowlands (D) landrace.

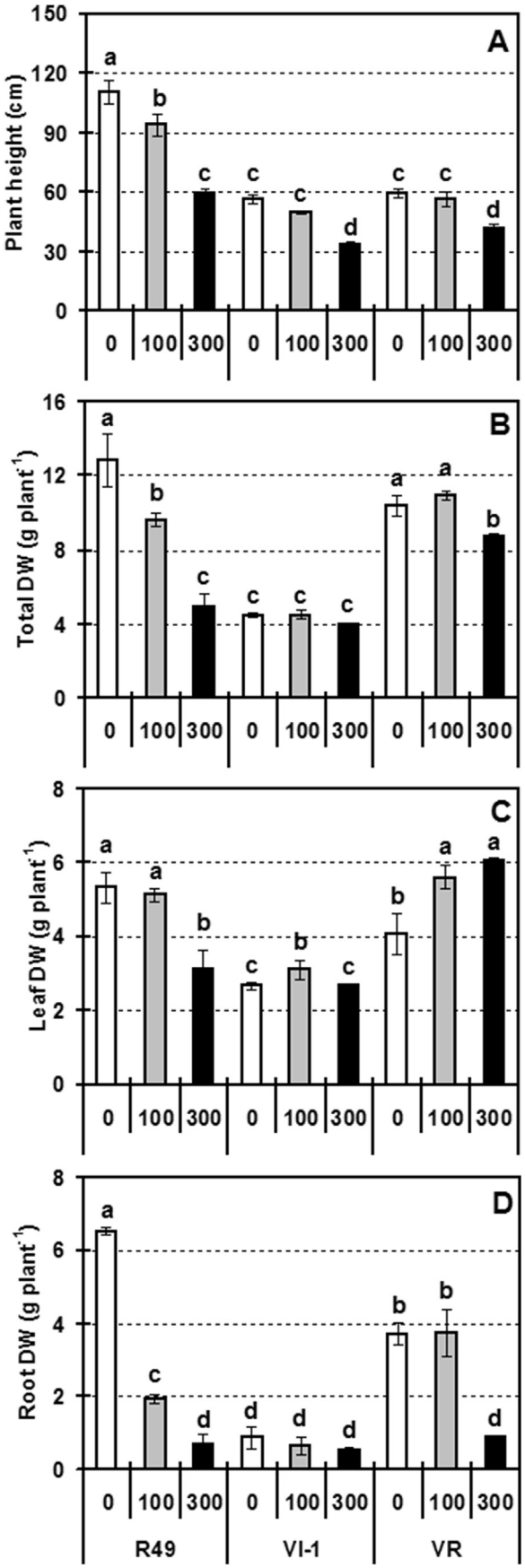

Fig. 3. Total plant height (A), total plant DW (B), leaf DW (C), and root DW (D) at the end of the experiment in the three C. *quinoa* landraces irrigated with 0, 100 or 300 mM NaCl. Data are the mean of three biological replicates (±SE). Letters above the bars indicate significant differences ($P < 0.05$) by two-way ANOVA (genotype x treatment).

Both doses of NaCl decreased flavonoid accumulation relative to controls in VI-1 (up to ca. 60%) and VR (up to ca. 40%), but not in R49 (Table 2).

3.5. Seeds

3.5.1. Yield

Seed production in untreated plants varied between 3.4 and 4.5 g plant^{-1} without significant differences between the three landraces (Fig. 4A). Interestingly, T1 improved seed production in VR, whereas it did not affect it in VI-1 and slightly but significantly inhibited it in R49. Under T2, yield in VR returned to control values, but was deeply impaired in R49 (80% inhibition) and to a lesser extent in VI-1 (40% inhibition).

Yield in terms of seed FW (Fig. 4B) and DW (Fig. 4C) was also evaluated in the three landraces. Both FW and DW of seeds from control plants of R49 were slightly higher than those of VI-1 while VR had the smallest seeds. Under T2, differences between landraces were also quite evident, with R49 having the lowest, and VI-1 the highest FW and DW (Fig. 4B, C). Indeed, R49 grown on 300 mM NaCl exhibited a four- and three-fold decline in seed FW and DW, respectively relative to controls; a small but significant decrease (30% less than controls) also occurred in VR, while VI-1 was not affected.

3.5.2. Germination

Seed obtained from control and salt-grown plants of the three selected landraces were *in-vitro* germinated in the presence of increasing NaCl concentrations (0, 100, 300 and 500 mM) in order to check for a "carry-over" or adaptation effect deriving from the saline or non-saline conditions under which the mother plants were grown. Seeds collected from non salt-treated plants of all landraces showed 88–100% germination on medium containing 0 mM NaCl (Fig. 5A, C, E). While medium containing the two lower concentrations of NaCl (100 and 300 mM) did not affect germination percentage of seeds from control plants of R49, the highest concentration (500 mM) reduced it to 45%. Seeds from control plants of VI-1 exhibited a lower (70%) germination on 300 mM NaCl than in the absence of salt, and only 20% germination on 500 mM NaCl. Seeds from control plants of VR had the lowest germinability (20% with 300 mM and 0 with 500 mM NaCl).

In general, seeds from plants irrigated with 100 mM NaCl showed a similar trend in response to increasing concentrations of NaCl as those from control plants (Fig. 5A, C, E). However, in R49, germination on 500 mM NaCl was slightly higher (67%) than that of seeds from control plants and in VI-1; it was significantly improved on both 300 mM and 500 mM NaCl (*ca*. 40% and 85% higher than seeds from control plants, respectively).

Seeds collected from R49 plants irrigated with 300 mM salt germinated significantly less than those from control and 100 mM NaCl-grown plants on 300 mM NaCl; on medium containing 500 mM NaCl, however, germinability was no different from control seeds but lower than that of seeds from plants grown with 100 mM salt (Fig. 5A, C, E). In VI-1 there was a slight improvement relative to control seeds at 300 and 500 mM NaCl (24% and 50% higher, respectively), whereas seeds of VR exhibited a strongly reduced germinability in the presence of salt (only 20% and 0% on 100 and 300 mM NaCl, respectively). In general, the best performing landrace was R49 insofar as 45–65% of seeds (from C, T1

Table 2
Chlorophyll *a* (Chla) and *b* (Chlb), total carotenoid (Cc+x), total polyphenol (TPC) and total flavonoid (FL) concentrations (all expressed in mg g^{-1} FW) in leaves of three landraces of C. *quinoa* grown on soil irrigated with 0, 100 or, 300 mM NaCl. Values are means of six independent extractions ± SE. Values followed by different letters are significantly different ($p < 0.05$; $n = 6$) by two-way ANOVA (genotype x treatment).

	NaCl (mM)	Chl*a*	Chl*b*	Cc + x	TPC	FL
R49	0	2.88 ± 0.34^{b}	0.40 ± 0.09^{a}	0.76 ± 0.07^{a}	7.70 ± 0.44^{b}	5.95 ± 0.56^{b}
	100	2.44 ± 0.25^{b}	0.43 ± 0.11^{a}	0.66 ± 0.06^{b}	10.85 ± 0.67^{a}	6.27 ± 0.89^{b}
	300	2.27 ± 0.08^{b}	0.38 ± 0.08^{a}	0.59 ± 0.04^{b}	11.43 ± 1.16^{a}	5.72 ± 0.66^{b}
VI-1	0	3.90 ± 0.46^{a}	0.71 ± 0.13^{a}	1.03 ± 0.10^{a}	9.89 ± 1.02^{a}	11.54 ± 1.44^{a}
	100	2.41 ± 0.18^{b}	0.50 ± 0.10^{a}	0.65 ± 0.06^{b}	6.07 ± 0.75^{c}	6.08 ± 0.99^{b}
	300	1.84 ± 0.21^{b}	0.39 ± 0.08^{a}	0.51 ± 0.07^{b}	4.26 ± 0.79^{c}	4.88 ± 0.76^{b}
VR	0	3.62 ± 0.75^{a}	0.76 ± 0.16^{a}	0.93 ± 0.11^{a}	9.67 ± 0.40^{a}	12.19 ± 1.80^{a}
	100	2.83 ± 0.28^{b}	0.54 ± 0.10^{a}	0.84 ± 0.05^{a}	5.43 ± 0.54^{c}	8.21 ± 1.08^{b}
	300	3.27 ± 0.41^{b}	0.57 ± 0.09^{a}	0.89 ± 0.08^{a}	5.17 ± 0.48^{c}	7.00 ± 0.54^{b}

and T2 plants) germinated on 500 mM NaCl-containing medium.

3.5.3. Seedling growth

Seedling growth varied markedly between landraces and depending upon the saline or non-saline treatment of the plants. On control medium (0 mM NaCl), roots of R49 seedlings were, on average, about twice as long as those of the other two landraces (Fig. 5B, D, F). Also at 500 mM NaCl, R49 roots (from C, T1 and T2 plants) were, on average, significantly longer (2.8 ± 0.9 mm) than those of VI-1 (1.1 ± 0.6 mm) and VR. On control medium, VI-1 and VR seedlings had shorter roots when seeds came from T2 plants but longer ones from T1 plants, whereas R49 roots from C, T1 and T2 plants had much more similar lengths (Fig. 5B, D, F). In all three landraces, average root length declined strongly with increasing NaCl concentration irrespective of the previous treatment. However, whereas in the presence of 100 mM NaCl root length of VI-1 and VR seedlings from T2 plants declined significantly relative to C plants, in R49 it increased (from an average of 30 mm–45 mm); this increase (T2 versus C/T1) remained, albeit it to a lesser extent, at 300 mM NaCl (5.3 ± 0.8 mm vs 0.2 ± 0.1 mm T2 vs C plants, respectively). Hypocotyl length followed a similar trend, with VI-1 and VR, but not R49, seedlings from T2 plants being more negatively affected by mildly saline media (100 mM NaCl) than those from T1 and C ones (data not shown).

3.5.4. TPC and antioxidant activities

Seeds from control plants of all three selected landraces had a similar TPC. Seed TPC was affected by salinity only in two of the three landraces analyzed, namely R49 and VR (Table 3). In the former, T2 led to a ca. 45% increase in seed TPC, and in the latter landrace both salt concentrations caused a similar (55–60%) increase above control levels. Thus, after irrigation with 300 mM NaCl, seeds of R49 and VR had a significantly higher TPC than those of VI-1. The radical scavenging capacity of seed extracts evaluated by the DPPH test revealed a significantly higher activity in seeds from 300 mM NaCl-treated plants of R49, but not in those of the other landraces (Table 3). The FRAP-FZ test confirmed the higher antioxidant capacity of R49 seeds from salt-treated plants as compared with control ones.

3.5.5. Protein content and profile

Total SDS-extractable protein concentration averaged 58.4 ± 0.58% in seeds of control plants without significant differences between the three selected landraces (Table 3). A slight but significant reduction (7–12%) in total protein concentration was observed in seeds from salt-treated plants relative to controls in all three landraces (Table 3). To investigate the effect of NaCl on the protein patterns of these seeds, total proteins were extracted and subjected to one-dimensional SDS–PAGE. As shown in Fig. 6A, the proteins were resolved into distinct bands that spanned a broad range of apparent molecular weights from 10 kDa to more than 70 kDa with some evident differences in the band patterns between landraces One major band of 25 kDa was present in all three landraces, but, in control plants, the intensity of this band was highest in VR, followed by VI-1 and R49. Some minor bands of about 30 kDa and 20 kDa were evident; in R49 the former was more evident than in the other two landraces. Results also showed that increasing NaCl concentrations produced significant changes in the protein patterns and that the three landraces were differentially affected by salinity (Fig. 6B). In R49, the 25- and 20-kDa bands were more intense in seeds treated with 100 mM NaCl as compared with controls and 300 mM NaCl-treated ones. In VI-1, a strong increase in the intensity of the 25-kDa band was observed in seeds from plants grown with the highest concentration of NaCl, while in control and T1 seeds the profile was very similar. Finally, in VR seeds, both salt treatments enhanced the intensity of the major 25-kDa band relative to controls.

4. Discussion

Hariadi et al. (2011) established that NaCl concentrations of 100–200 mM (approx. 10 to 20 dS m^{-1}) were optimal for quinoa growth. In our study, plants were irrigated with relatively low doses of NaCl compared with other experiments where saline solutions of up to 750 mM have been applied. However, soil conductivity reached average values of *ca.* 32 and 51 dS m^{-1} after repeated irrigation with 100 mM (T1) and 300 mM (T2) NaCl, respectively at the end of the experiment. Therefore, quinoa plants in our experiment were exposed to very high salinity compared to previous work. Overall, present results corroborate the notion that growth, yield, and physiological parameters are differentially affected by salinity in the quinoa landraces examined, even those belonging to the same ecotype. However, R49, the salt flats landrace, could be distinguished from the coastal lowlands landraces.

4.1. Leaf Na^{+} accumulation distinguishes the three landraces

Leaf and root [Na^{+}] increased dramatically with NaCl application, albeit with significant differences between landraces. Under T2, [Na^{+}] was up to *ca.* 41-fold and 38-fold control levels in roots and leaves, respectively. These results suggest that quinoa's salt tolerance is not based on an exclusion mechanism, i.e., limited ion influx. Indeed, high accumulation of ions (Na^{+}, Cl^{-}, K^{+}) in leaves of quinoa has been reported earlier (Orsini et al. 2011) and the importance of these inorganic ions for osmotic adjustment has been emphasized (Hariadi et al. 2011). With 300 mM NaCl, VI-1 exhibited the highest leaf and root [Na^{+}] so that, on the whole, it was the genotype that accumulated the most in plant organs. The highest root [Na^{+}] under T2 was observed in R49, the *salares* ecotype from the *altiplano* of northern Chile, and in VR, a coastal

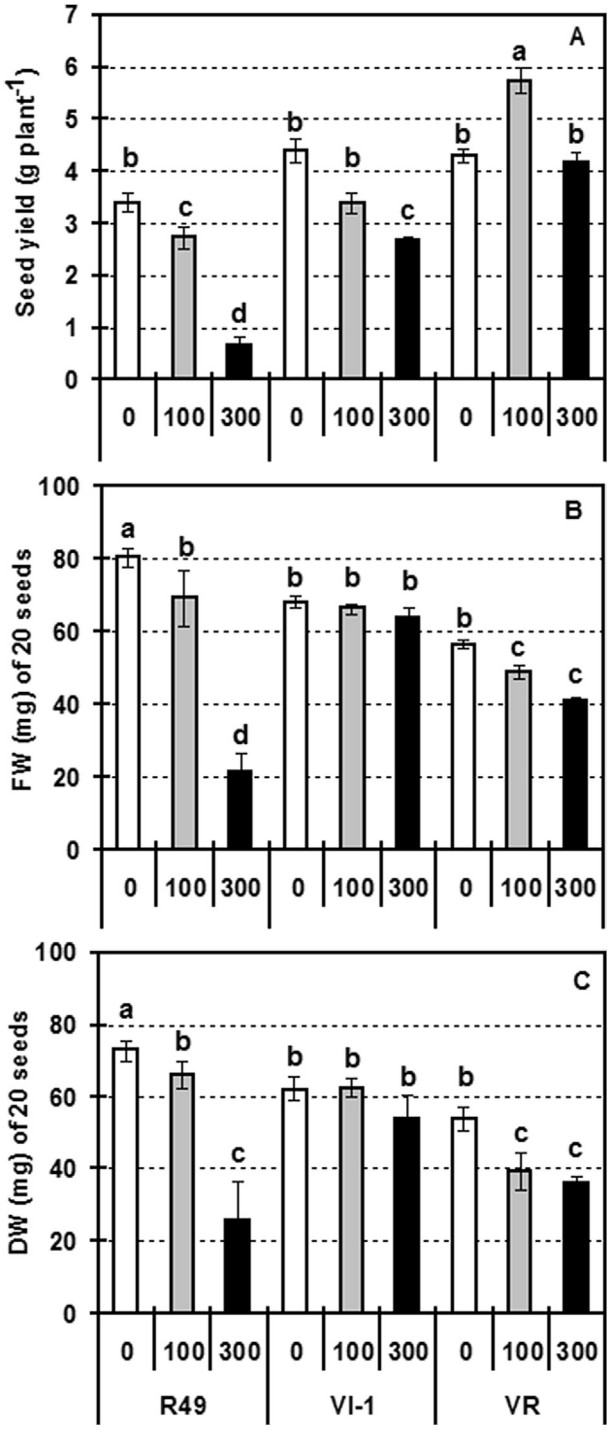

Fig. 4. Seed yield per plant (A), seed FW (B) and seed DW (C) from plants of the three landraces of C. *quinoa* grown on soil irrigated with 0, 100 or 300 mM NaCl. Values are means of three replicates (±SE) per plant. Letters above the bars indicate significant differences ($P < 0.05$) by two-way ANOVA (genotype x treatment).

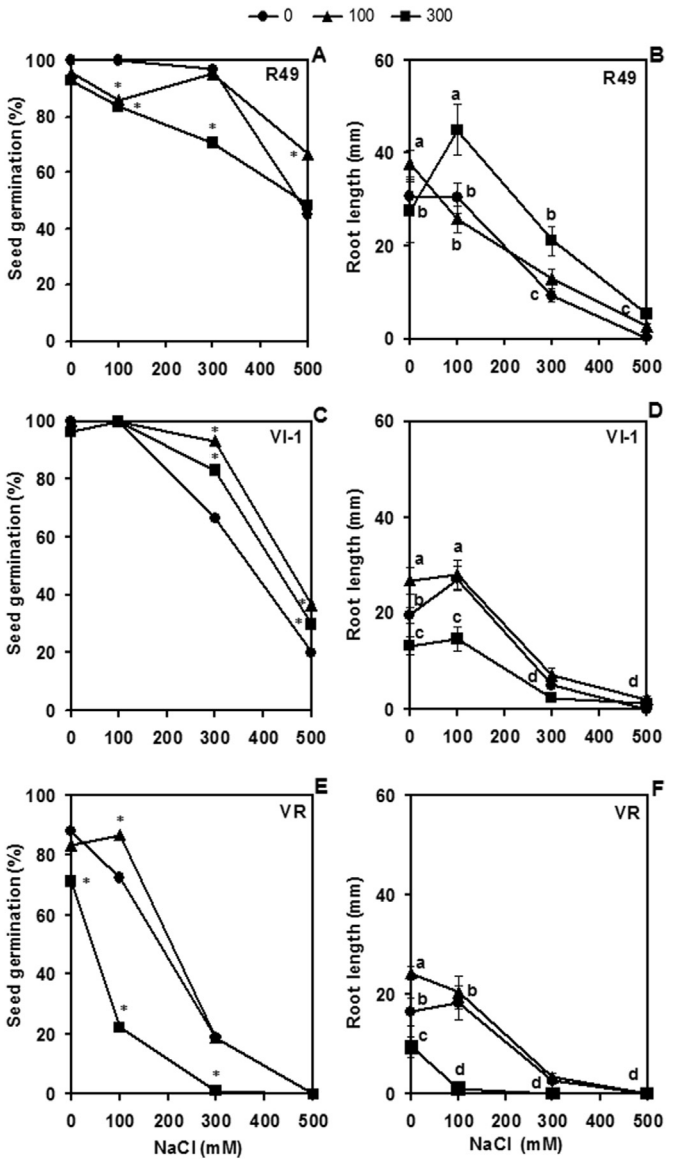

Fig. 5. *In vitro* seed germination (A, C, E) and seedling root length (B, D, F) 10 days after sowing on media supplemented with 0, 100, 300 and 500 mM NaCl. Seeds were collected from landraces of C. *quinoa* grown on soil irrigated with 0, 100 or 300 mM NaCl. Values are means of three replicates per plant (±SE). Asterisks indicate significant differences ($P < 0.05$) by the χ^2 test (percent germination) and letters indicate significant differences ($P < 0.05$) by two-way ANOVA (root length).

ecotype from southern Chile, therefore, this parameter could not distinguish tolerance based on original habitat. Genotypic differences in leaf-to-root [Na^+] ratio indicated which landrace was less apt to accumulate this ion at the foliar level. Both under T1 and T2, R49 exhibited the lowest ratio, suggesting that it is an "excluder", where exclusion is defined as low accumulation in leaves (Shabala et al. 2013) and not low uptake at the root level. This relatively low Na^+ translocation to the leaves may be regarded as a salt tolerance mechanism. It may be a way of avoiding ion toxicity in the photosynthetic organs; in fact, chlorophyll content was not severely affected by salt in R49 whereas it was negatively affected in VI-1 which had the highest leaf [Na^+]. Previous studies have shown that Na^+ was preferentially accumulated in quinoa shoots, perhaps because quinoa is more susceptible to ion toxicity in roots than in above-ground organs (Panuccio et al. 2014). Ion toxicity in the roots of R49 could not be avoided under T2 and this was probably reflected in strongly impaired growth (*ca.* three-fold decrease in dry biomass and two-fold decrease in height) and seed production.

4.2. Salt effects on plant biomass and phenology distinguish the salares landrace

Plant height and dry weight are generally affected by severe salt

Table 3
Functional characteristics of seeds harvested from plants of three Chilean landraces irrigated with 0, 100 or 300 mM NaCl. TPC, total phenolics content. TAC, total antioxidant capacity, as assayed by the DPPH and FRAP-ferrozine methods and expressed as μM Trolox equivalents g^{-1} DW. Values are means ± SE (n = 6). Values followed by different letters are significantly different ($p < 0.05$; n = 6) by two-way ANOVA (genotype x treatment).

	mM NaCl	TPC (mg GAE g^{-1} DW)	TAC (DPPH)	TAC (FRAP)	Protein concentration (mg 100 mg^{-1} DW)
R49	0	1.14 ± 0.11^{b}	0.56 ± 0.04^{b}	0.16 ± 0.03^{b}	57.6 ± 2.2^{a}
	100	1.05 ± 0.15^{b}	0.64 ± 0.04^{b}	0.18 ± 0.01^{b}	54.2 ± 0.7^{b}
	300	1.64 ± 0.35^{a}	0.86 ± 0.08^{a}	0.37 ± 0.04^{a}	50.6 ± 2.0^{b}
VI-1	0	1.16 ± 0.10^{b}	0.99 ± 0.10^{a}	0.27 ± 0.04^{a}	58.7 ± 2.1^{a}
	100	1.09 ± 0.15^{b}	0.81 ± 0.15^{a}	0.24 ± 0.01^{a}	52.1 ± 2.6^{b}
	300	1.18 ± 0.21^{b}	1.09 ± 0.14^{a}	0.26 ± 0.03^{a}	54.4 ± 1.0^{b}
VR	0	1.27 ± 0.07^{b}	1.22 ± 0.08^{a}	0.27 ± 0.01^{a}	58.4 ± 1.2^{a}
	100	1.98 ± 0.30^{a}	1.43 ± 0.26^{a}	0.31 ± 0.05^{a}	52.3 ± 0.4^{b}
	300	1.99 ± 0.18^{a}	1.39 ± 0.03^{a}	0.34 ± 0.01^{a}	54.4 ± 0.2^{b}

treatments even in halophytes, and are regarded as useful traits to distinguish degrees of salt tolerance in quinoa landraces (Gómez-Pando et al. 2010; Ruiz-Carrasco et al. 2011). Adolf et al. (2012) reported strongly genotype-dependent responses to 400 mM NaCl with percentage decreases in biomass varying from ca. 18–50% and ca. 8–35% for height. In the present study, height was negatively affected by 300 mM NaCl in all three landraces, but the range of genotypic variation in height decrease in T2 versus controls was fairly restricted, with the strongest inhibition in R49. By contrast, total plant biomass was practically unaffected in the coastal lowlands landraces, but severely reduced in R49. As suggested by Adolf et al. (2012), height was not a good predictor of biomass for VI-1 and VR. Although Bosque-Sánchez et al. (2003) observed that total plant biomass of the "Real" variety of quinoa, native to the Bolivian *altiplano*, was not negatively affected by irrigation with water having an EC up to 20 dS m^{-1}, in our study R49 displayed reduced total dry biomass both under T1 and T2 and was more negatively affected than the other landraces. Root dry mass per plant was negatively affected by T2 in R49 and VR, whereas leaf dry mass was enhanced relative to controls, except in R49. Thus, in R49, total plant DW was strongly reduced by T2 due to a reduction in root biomass, while in the other landraces total biomass remained unaltered either because root dry biomass was scarcely affected (VI-1) or because leaf dry biomass increased (VR). Moreover, VI-1, which, under T2, had the highest foliar concentration of Na^{+} also displayed enhanced leaf succulence (i.e., higher FW per unit leaf area, data not shown). No correlation was observed between foliar [Na^{+}] and plant biomass except for a strong negative correlation in R49 ($r^2 = 0.76$); the highest negative correlation between root [Na^{+}] and total biomass was also observed in R49 ($r^2 = 0.78$). Gómez-Pando et al. (2010) compared 182 Peruvian genotypes of quinoa under salinity and showed that genotype and salt treatment strongly influenced root dry mass per plant more than height. The general reduction in root biomass is also consistent with data by Panuccio et al. (2014) who showed that 21-day old quinoa seedlings irrigated with 50% seawater had significantly lower root lengths, surface areas, and volumes than controls.

Plant morphology can also change under salinity. A feature of drought/salt tolerant plants is that of having a root system which penetrates the soil in depth either as an avoidance mechanism or to find water. Alvarez-Flores et al. (2014) compared plant growth and root morphology in two *C. quinoa* landraces, one from the temperate lowlands with nutrient-rich soils (Chile) and one from the arid cold Bolivian *altiplano* with nutrient-poor soils. Quinoa from the low-resource habitat had faster main root growth. The *salares* landrace R49 does, in fact, have a longer main root (but less branched root system) than other genotypes (Fig. 2B,D). This was confirmed by data on root length of seedlings grown *in vitro* on saline and non-saline media. Shorter plants, such as those observed here under salinity, exhibit more compact growth. Some varieties can become thinner, while others have a different number of internodes (Adolf et al. 2012), or different internode lengths, a feature observed in our study. Different internode length leads to a different distribution of leaves. Yield will be affected by all of these changes in morphology as a consequence of differences in light distribution within the canopy. As regards phenology, Gómez-Pando et al. (2010) reported that quinoa genotype and salt treatment did not noticeably affect the length of the plant's life cycle. Present results show that salinity did not substantially affect harvest time, but some phenological stages were delayed or shortened with visible differences again between R49 and the coastal landraces.

4.3. Physiological parameters indicate that R49 exhibits protective mechanisms

Under saline conditions, chlorophyll content generally decreases in salt-sensitive plants whereas in salt-tolerant ones increases in chlorophyll content have been observed (Khan et al. 2009). In a comparison between two quinoa varieties with highly contrasting origins, Utusaya from the Andean highlands close to the Bolivian salt flats, and Titicaca a cultivar selected in Denmark under non-saline conditions, Adolf et al. (2012) reported that while chlorophyll fluorescence (Fv/Fm ratio) was not significantly affected in either of the two genotypes, chlorophyll content increased in Utusaya and decreased in Titicaca. Our data support the inherent potential of the *salares* landrace R49 to tolerate salinity insofar as it was the only one of the three genotypes analyzed not to exhibit decreased photosynthetic pigment concentrations either under T1 or T2. Maintaining or even enhancing chlorophyll and carotenoid levels under saline conditions may be considered a desirable trait because it indicates a low degree of photo-inhibition (Sheshshayee et al. 2006). However, both osmotically-induced stomatal closure and Na^{+} toxicity can impair the plant's capacity to fully utilize light absorbed by the photosynthetic pigments, leading to the formation of reactive oxygen species (ROS) (Shabala et al. 2012). Although halophytes may use the enzymatic antioxidant machinery more efficiently than glycophytes (Srivastava et al. 2015), non-enzymatic antioxidant compounds also play a crucial role. Amongst these, polyphenols have a strong ability to scavenge ROS; of the several thousand polyphenols known in plants, flavonoids form the largest group. A more effective use of polyphenols and higher antioxidant activities in halophytes as compared with glycophytes has been reported (Bose et al. 2014). Polyphenols, flavonoids, and antioxidant activities have been determined in seeds of quinoa (Gómez-Caravaca et al. 2012; Panuccio et al. 2014) but not in leaves of salt-treated plants. Present results indicate that, under salinity, R49 was the only landrace to exhibit significantly enhanced TPC and unaltered flavonoid concentrations in leaves as opposed to the other landraces where both classes of compounds decreased

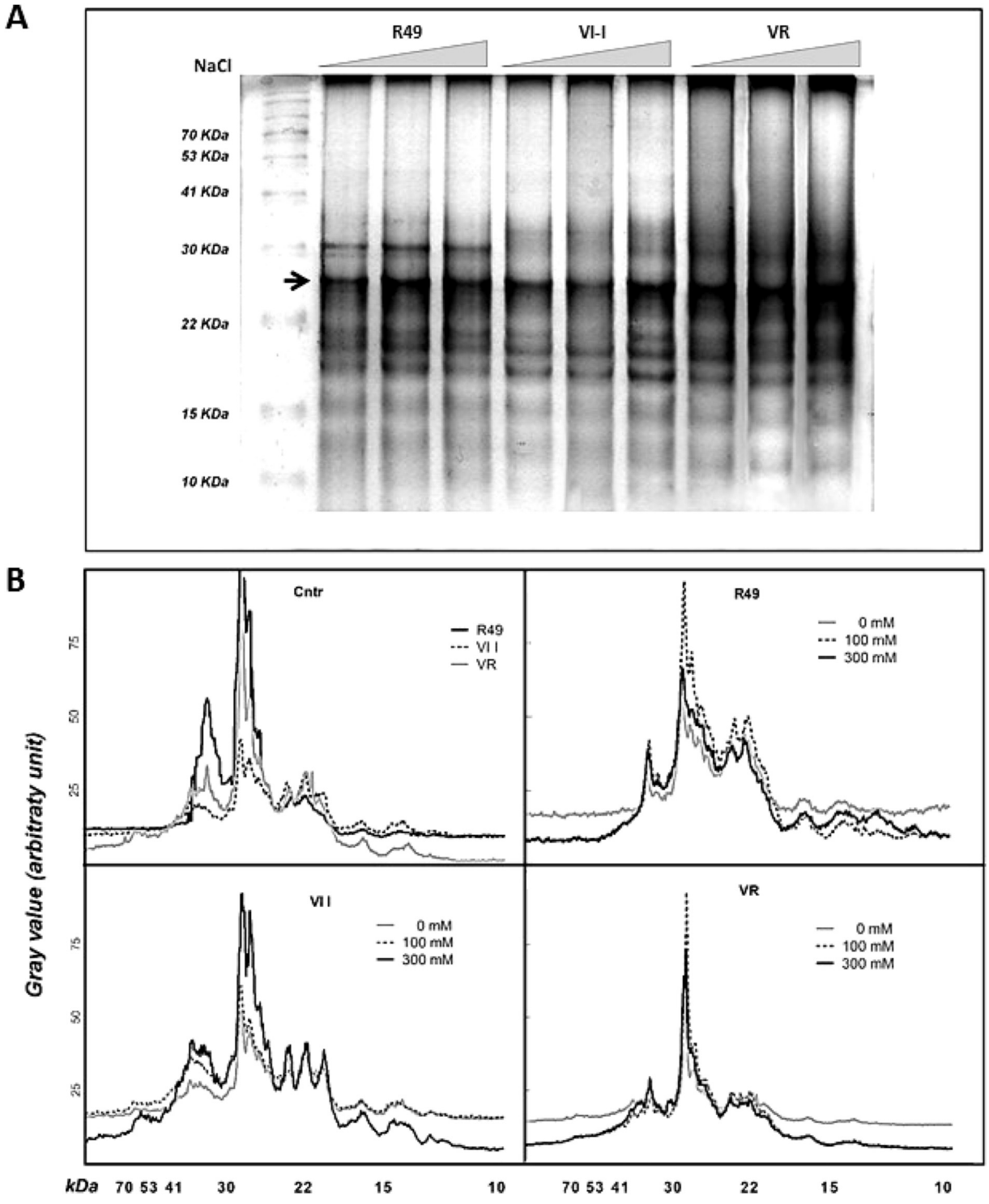

Fig. 6. SDS-PAGE analysis of total seed proteins of quinoa landraces R49, VI-I, and VR. (A) SDS-PAGE gel. Forty μg of the seed protein extract was loaded in each lane. Standard molecular weights are shown on the left. (B) Lane profile plots, based on electrophoretic densitometry, of non-salt treated samples of the three landraces (Cntr) and samples of each landrace grown with 0, 100, and 300 mM NaCl. Values are arbitrary units.

markedly. This feature can possibly be related to the need, in R49, to mitigate photo-oxidative damage generated by diminished photosynthetic rate (reduced stomatal conductance; Razzaghi et al. 2011; Orsini et al. 2011) in the face of undiminished photosynthetic pigment contents. Taken together, these parameters support the higher potential salt tolerance of the landrace from the more extreme habitat (*salares* ecotype) from a physiological point of view, although final performance (growth, yield) was negatively affected under the experimental conditions applied here. This "trade-off" between growth and resistance was previously observed in a comparison between the *salares* genotype Utusaya and the Danish-bred cv. Titicaca (Adolf et al. 2012).

4.4. Seed yield is reduced but germination and seedling growth is better in R49 under salt stress

Although the Peruvian quinoa cv. Hualhuas was able to complete its life cycle even at salinity levels as high as 500 mM NaCl, seed production was strongly reduced even under moderate salt treatments (Koyro and Eisa, 2008). Razzaghi et al. (2011) likewise reported a significant reduction in seed yield under all the salinity levels tested (10–40 dS m^{-1}) in cv. Titicaca. In Peruvian germplasm, seed yield was, depending on the genotype, unaffected, strongly reduced or enhanced under 30 dS m^{-1}; the reduction in grain yield under salt stress ranged from 4.8 to 81.3% (Gómez-Pando et al. 2010). In a comparison between Chilean lowland cultivars, Peterson and Murphy (2015) reported seed yield declines at 32 dS m^{-1} compared with the non-saline control ranging from 43.8 to 73.7%.

Since height and biomass are closely related to productivity, the strong reduction observed in R49 seed production under salinity as compared with the other landraces is not surprising. Thus, a strongly positive correlation between seed yield (g $plant^{-1}$) and total plant biomass was observed in R49 ($r^2 = 0.83$). The two coastal lowlands landraces, though less strongly affected by high salinity, could be distinguished in terms of seed yield and size (FW and DW) with VR exhibiting a higher sensitivity than VI-1. Relative declines in seed FW and DW due to salinity for the coastal lowland landraces thus increased going southward (i.e., higher latitudes of origin). Despite the strongly reduced seed size of salt-treated R49 plants, their germination capacity on NaCl-supplemented media was less impaired than in VI-1 and VR (see below).

It is a general observation that, despite strong genotypic differences, seed germination is inhibited by salinity in quinoa, as in other halophytes (Karyotis et al. 2003). Percent germination and germination rate (or both) are regarded as an effective and rapid method to distinguish tolerant from less tolerant genotypes (Delatorre-Herrera and Pinto, 2009; Ruiz-Carrasco et al. 2011). Present results indicate that R49 had the highest germination capacity under high salinity compared with the coastal lowlands landraces. Based on this parameter too, R49 appears to display a higher adaptive potential to saline conditions. Moreover, R49 seedlings exhibited a better growth response to saline media with significantly higher root and hypocotyl length than VI-1 and VR. VI-1 from central Chile (*secano costero*) could be distinguished from VR (from the humid zone) based on a stimulation of germination and seedling root length by 100 mM NaCl and a lower inhibition by the higher NaCl levels in the former. Thus, seed size, seed germination, and seedling growth on saline media appear to be the only parameters that enable to distinguish between landraces of the coastal lowlands ecotype, with VR exhibiting a higher sensitivity to salt than VI-1. In fact, it should be pointed out that VI-1 originates from coastal lowlands but is grown in marine terraces with high salt content, unlike VR, which comes from non-saline areas.

In order to address the question as to whether or not plants develop higher salt tolerance after they are exposed to saline conditions, Koyro and Eisa (2008) tested the germination rate on 0–500 mM NaCl solutions of seeds harvested from plants grown with different salt concentrations. Although some differences in germination rates were observed, final percentages (day 8) were all similar, but significantly lower for seeds from plants grown with 500 mM. In the present study, R49 seeds maintained the highest germination capacity on strongly saline medium (500 mM) regardless of the mother plants (C, T1 or T2). Moreover, in R49 and VI-1, plant irrigation with 100 mM NaCl enhanced percentage germination on strongly saline media (300/500 mM) as compared to irrigation with 0 mM, suggesting that seeds from salt-treated plants may have acquired enhanced salt tolerance. Similarly, R49 seedlings from T2 plants behaved very differently as compared with VI-1 and VR ones insofar as seedling root growth was enhanced, relative to C and T1 plants, at all salinity levels. VR seeds exhibited the highest sensitivity to saline media but also to irrigation of the mother plants with 300 mM NaCl. In this case, exposure of plants to high salinity appears to have impaired tolerance to salt during the initial stages of plant establishment in the next generation.

4.5. Seed quality is maintained or improved under saline conditions

Despite being a crop that is attracting the attention of researchers and consumers worldwide because of the nutritional properties of its seeds, relatively few studies have dealt with the effects of salinity on seed quality. Panuccio et al. (2014) reported that TPC and total antioxidant capacity of quinoa seeds increased, relative to controls, three days after sowing directly on saline media. Miranda et al. (2013) compared two Chilean genotypes grown under contrasting environments, arid and cold-temperate. Results showed that in the former location phenolic compounds and components of proximate analysis (except protein content) increased. Present results show that both R49 and VR had higher TPC in seeds harvested from plants grown on saline soils relative to controls. This response can be interpreted as a protective mechanism in the case of R49 (reflected in better germinability) but not VR. At the same time, it enhances the nutritional properties of seeds. In accord with their increased TPC, seeds from R49 plants grown under T2 also exhibited enhanced TAC (radical scavenging and reducing potential) as compared with seeds from control plants. This too represents an added health-benefit value.

Although quinoa's nutritional properties are to a large extent related to its protein-rich seeds (Vega-Gálvez et al. 2010), there is little information concerning seed protein content or quality under stress conditions. Abugoch et al. (2008) reported, for seeds dried until 15% moisture, a protein content of ca. 77–84%. In flour obtained from oven-dried seeds we found similar levels of total SDS-soluble proteins. Total soluble protein content was not significantly different in the three landraces and was only very slightly reduced by T1 and T2. This is in contrast to previous studies reporting that salinity led to an increase of the protein concentration in quinoa seeds, possibly as a strategy to improve seed germination/seedling establishment (Koyro and Eisa, 2008). The majority of the European and South American varieties analyzed by Karyotis et al. (2003) also accumulated significantly more protein under saline-sodic soil conditions. The small decrease in protein content under salinity observed in our experiment should not be significant with respect to the nutritional potential of the seeds. A preliminary analysis of the total seed proteins by one-dimensional SDS-PAGE revealed that the protein profile in the three genotypes were similar even though the major band (25-kDa) was much less intense in R49 and VI-1 than in VR. Protein profiles were also differentially affected by T1/T2 in the three landraces. Thus, although total protein content decreased slightly under salinity, some bands increased and, therefore, these proteins need to be further characterized in order to assess: a) whether they represent another adaptive mechanism (e.g., dehydrin accumulation in seeds has been proposed as a salt-tolerance mechanism; Burrieza et al. 2012), and b) what implications they may have on the nutritional value of quinoa seeds.

5. Conclusions

All parameters measured were differentially affected in a genotype-dependent way. Seed size as well as germination and seedling growth on saline media could be suitable for screening among the coastal lowlands Chilean landraces from the drier and

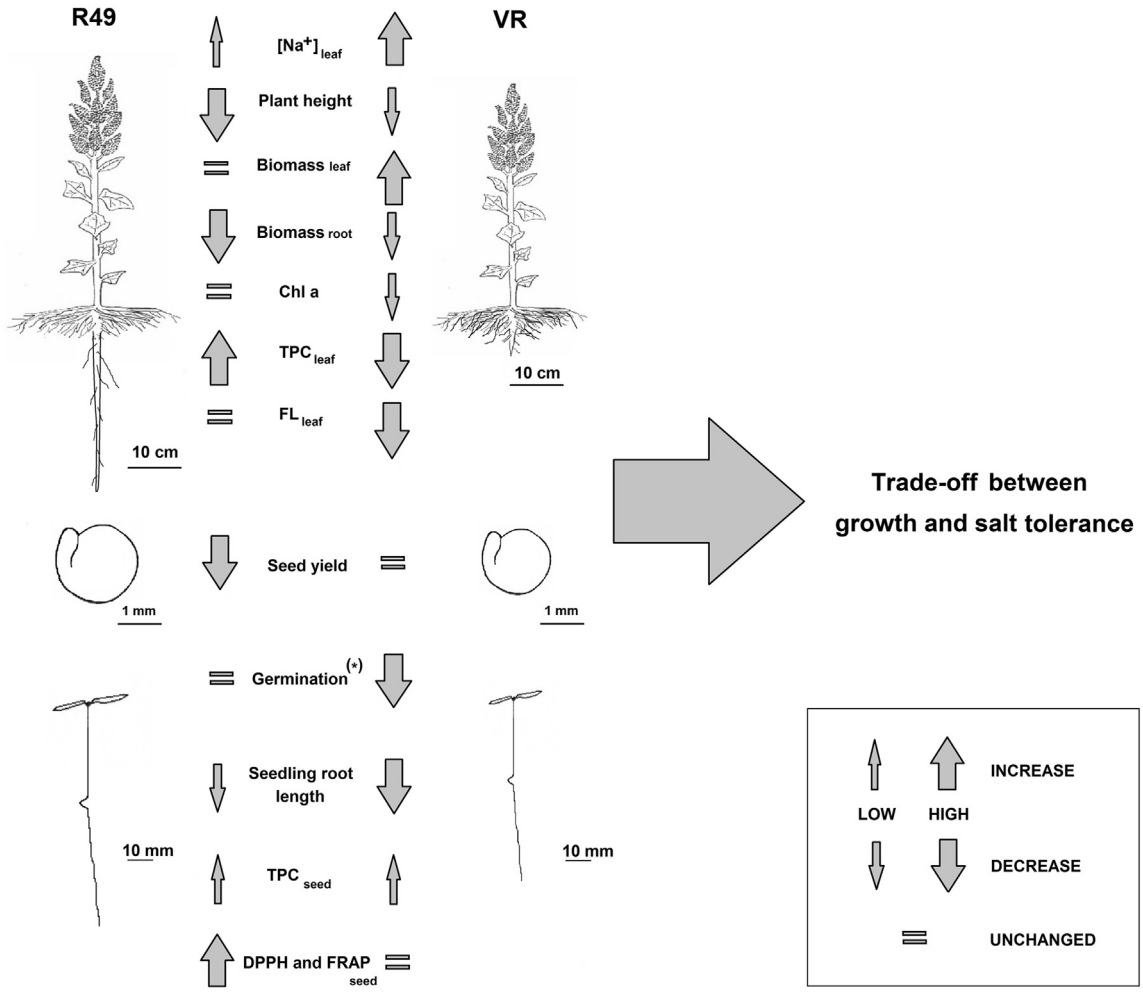

Fig. 7. Schematic representation of the main features distinguishing the *salares* landrace R49 from the coastal lowlands landrace VR. Symbols (arrows) indicate changes in 300 mM NaCl treatments relative to controls (0 mM NaCl). (*), seeds harvested from control plants sown on medium containing 300 mM NaCl.

more humid zones. Unexpectedly, R49 did not respond positively under salinity in terms of growth (height, biomass) and yield, thus these were not useful parameters for establishing the higher tolerance of the *salares* landrace relative to the other two. There were, however, major differences between R49 and the coastal lowlands landraces in a number of other parameters likely representing tolerance mechanisms, namely: Na^+ exclusion (relative to other genotypes) from leaves, longer roots, no changes in photosynthetic pigments, enhanced TPC and flavonoids in leaves, enhanced TPC and antioxidant activities in seeds, accompanied by superior ability to germinate under saline conditions (Fig. 7). Thus, under our experimental conditions (which were perhaps not optimal in terms of photoperiod and irradiance for R49), the *salares* landrace from the northern *altiplano* of Chile did not display higher tolerance to salinity in terms of height, biomass and seed yield despite its original habitat. This may reflect a negative correlation between plant adaptive potential and productivity or the well-known "trade-off" between stress resistance and growth or yield or both. The landraces belonging to the coastal lowlands ecotype, on the other hand, revealed interesting responses to salinity in terms of growth, yield, and seed quality (TPC, protein profiles), which deserve further investigation.

Contributions

KBR and SB conceived and designed the experiments. KBR and VC conducted the experiments, collected the agronomic and physiological data. IA and SDD performed the protein analyses. HS provided the facilities and advised on the preparation of plant materials. KBR, SB and PT contributed to elaborate the data and write the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2016.01.010.

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