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Comparing salt-induced responses at the transcript level in a *salares* and coastal-lowlands landrace of quinoa (*Chenopodium quinoa* Willd)

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ABSTRACT

To further our understanding of the mechanisms governing salt stress responses and adaptation in halophytes, we explored morphological, metabolic, and gene expression responses to high salinity in quinoa (*Chenopodium quinoa* Willd). The main objective of this study was to analyze selected responsive genes in a time-course experiment to test for expression kinetics and to compare short-term salt-induced effects at the transcript level between two Chilean landraces belonging to different ecotypes. Quinoa genotypes exhibit a large variability in their responses to salinity, but it is not clear whether this is strictly related to the ecotype to which they belong. We tested this hypothesis by comparing the expression levels of genes involved in growth, ion homeostasis, abscisic acid (ABA) biosynthesis, perception, and conjugate cleavage, polyamine (PA) biosynthesis and oxidation, and proline biosynthesis as well as genes encoding ABA-dependent and −independent transcription factors. Landraces R49 (*salares* ecotype) and Villarrica (VR, coastal-lowlands ecotype) were analyzed from 0.5 to 120 h after transfer to saline (300 mM NaCl) or non-saline (control) medium. All the genes, except *CqSOS1* and *CqNHX*, were investigated here for the first time in quinoa under salt stress. Transcript levels were determined by quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) analysis. Germination, seedling growth, ABA, and PA contents were evaluated in parallel. Even though on saline medium germination was inhibited in VR but not in R49, seedling growth reduction at 120 h was not substantially different in the two landraces. The ABA biosynthetic enzyme *NCED* was the most strongly salt-induced gene; ABA content was similarly enhanced (shoots) or unaffected (roots) in both R49 and VR. NaCl treatment also altered transcript levels of some PA metabolic enzymes and the PA profile leading to an enhanced ratio between the higher PAs and putrescine. All other genes also exhibited similar expression profiles in response to salinity in the two landraces especially in roots, while in shoots some differences were observed. Our results provide new information indicating that crucial salt adaptation strategies at the molecular level and in terms of ABA and PA contents are shared by the coastal-lowlands and *salares* landraces; however, the timing of the onset of transcriptional changes (e.g., *NCED*, *ABF3*, and *RD22*) may reflect genotype-dependent constitutive and/or inducible adaptive strategies.

1. Introduction

The conditions under which crops can be cultivated are strongly influenced by global climate change, in particular by increasing aridity and soil salinity. Sustainable agriculture adapted to a changing climate and an increasing world population must rely on the use of suitable crop species, or genotypes within species, resistant to abiotic stresses and with good nutritional properties (Ruiz et al., 2014). Quinoa (*Chenopodium quinoa* Willd) is the only halophytic crop species producing edible seeds with highly nutritious properties (Vega-Gálvez et al., 2010; Ruiz et al., 2016b). The species' broad diversification in terms of native habitats accompanied by a high genetic diversity (Fuentes et al., 2009) has led to the identification of five ecotypes (Bazile et al., 2016). The *salares* ecotype is adapted to the extremely arid highland deserts in

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the *Altiplano* of the Andes (southern Bolivia, northern Chile, and northern Argentina). The coastal-lowlands ecotype is found in central and southern Chile where it can grow at sea level; here annual rainfall ranges from 400 to 1,500–2,000 mm and soils have a high water retention capacity. Strong differences in salt tolerance between quinoa varieties and landraces have been documented, both in terms of agronomic features and physiological responses (Adolf et al., 2012; Gómez-Pando et al., 2010; Ruiz et al., 2016a).

The reduction in plant growth under salinity is due to two main stress factors, osmotic and ionic (Hanin et al., 2016; Yamamoto et al., 2015). Consequently, some of the most important physiological mechanisms of salt tolerance in both glycophytes and halophytes are to a large extent based on ion homeostasis. The latter is principally guaranteed by Na^+ exclusion *via* limited root uptake or exclusion from photosynthetically active organs or both (Taji et al., 2004), cytosolic K^+ retention, and vacuolar Na^+ sequestration (Hanin et al., 2016), the latter being particularly important in halophytes that, different from glycophytes, achieve osmotic adjustment mainly through the accumulation, and not exclusion, of energetically inexpensive inorganic ions rather than low-molecular-weight organic solutes (Hariadi et al., 2011; Orsini et al., 2011; Bonales-Alatorre et al., 2013). Thus, genes encoding transporters that mediate Na^+ and K^+ homeostasis, such as *NHX*, Salt Overly Sensitive (*SOS),* and *AKT* are associated with salt tolerance (Apse and Blumwald, 2007; Albaladejo et al., 2017). In *Antirrhinum majus*, genes encoding cyclin-dependent kinases, various transcription factors, and ion transport proteins, as well as genes involved in abscisic acid (ABA) and ethylene signaling pathways were also differentially expressed in response to NaCl (Trivellini et al., 2016).

The ABA response to salt stress is one of the first committed steps leading to adaptation, *via* activating specific pathways and modifying gene expression levels (Ismail et al., 2014), including the gene encoding the key enzyme in ABA biosynthesis 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Geng et al., 2013; Dong et al., 2015). The expression of genes encoding enzymes involved in the degradation of the conjugated forms of ABA is also regulated by ABA and environmental stresses, including high salinity (Dong et al., 2015). In particular, free ABA levels during dehydration and osmotic stress can be modulated by the hydrolytic activity of β-glucosidase homologues (AtBG1 and AtBG2) on inactive ABA-glucose esters (ABA-GE) (Lee et al., 2006). ABA signals are perceived by multiple cellular receptors. The predominant type of ABA receptors is the PYRABACTIN RESISTANT1 (PYR1)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) localized in the cytosol and nucleus; *PYR*-related genes, designated *PYR-like* (*PYLs*), with varying affinities for ABA and other signalling components, have been identified (Finkelstein, 2013). Recently, phylogenetic analyses revealed that the *PYR/PYL/RCAR* gene family was substantially expanded in the quinoa genome compared with other Amaranthaceae (Yasui et al., 2016). ABA-BINDING FACTORS (ABFs) are basic leucine zipper (bZIP) domain transcription factors that bind ABA-RESPONSIVE PROMOTER ELEMENTS (ABREs) in the promoters of ABA-inducible genes (Dong et al., 2015). bZIP transcription factors are, therefore, involved in inducing downstream ABA-responsive gene expression and are among the target proteins of ABA core signalling (Shinozaki and Yamaguchi-Shinozaki, 2007). In *Arabidopsis*, the major *cis*-acting elements that function in an ABA-independent manner during abiotic stress responses are Dehydration-Responsive Element (DRE)/C-repeat (CRT; Finkelstein, 2013). Transcription factors that bind DRE and CRT elements are designated DRE-binding proteins (DREBs) and CRT-binding factor (CBF), respectively. *DREB2* genes are involved in responses to drought and high salinity (Sun et al., 2015; Shavrukov et al., 2016). Induction of drought-inducible *Dehydration Responsive Protein* genes are likewise mediated by ABA (e.g. *RD22*, *RD26*, *RD29*; Hanana et al., 2008; Nakashima et al., 2014).

The diamine putrescine (Put), and the higher polyamines (PAs) spermidine (Spd) and spermine (Spm), are important plant growth regulators involved in a wide range of biological processes (Kusano et al., 2008). PAs also play a major role in biotic and abiotic stress responses (Alcázar and Tiburcio, 2014). Under salt stress, they may function as osmolytes, scavenge stress-generated ROS, promote antioxidant activities, and act as signaling molecules in hormonal pathways (e.g., ABA); they also regulate redox homeostasis, and ionic balance *via* regulating plasma membrane and tonoplast proton pumps, as well as K^+ and non-selective cation channels, thereby improving vacuolar Na^+ sequestration and cytosolic K^+/Na^+ homeostasis (Minocha et al., 2014; Pottosin and Shabala, 2014; Pál et al., 2015; Saha et al., 2015). Ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) directly or indirectly catalyze the biosynthesis of Put, while Spd and Spm are synthesized from Put via Spd synthase (SPDS), and Spm synthase (SPMS), respectively, by the addition of the amino-propyl groups generated by S-adenosylmethionine decarboxylase (SAMDC). Put and PAs are catabolized by diamine oxidase (DAO) and polyamine oxidase (PAO), respectively. PA biosynthetic genes, especially *ADC2*, *SPDS1*, and *SPMS1*, are major stress-induced genes and their overexpression in several crop species confers enhanced tolerance to abiotic stress (Alcázar and Tiburcio, 2014; Do et al., 2014). In *Arabidopsis*, *AtDAO* gene expression is induced by wounding and involved in water balance homeostasis (Ghuge et al., 2015).

Genes likely to be involved in salinity tolerance, therefore, fall into three main functional categories: (1) those that regulate growth by controlling the rate of cell division (e.g., cyclin genes), expansion, and differentiation (2) those that control salt uptake, translocation, and compartmentalization; (3) those that have protective functions against environmental stresses (e.g. proline, PAs, ABA) (Munns, 2005). Although quinoa represents an interesting model species for studies on abiotic, in particular salt, stress responses (Ruiz et al., 2016b), genome-wide transcriptomic analyses for the response to salinity have been performed in several halophytes (Song and Wang, 2015; Wang et al., 2015; Yamamoto et al., 2015), but not in quinoa. To date, only the ion homeostasis genes *CqSOS1a* and *CqNHX* were cloned and their expression levels analyzed under high salinity (Maughan et al., 2009; Ruiz-Carrasco et al., 2011), but information regarding other genes implicated in salt stress responses is still lacking.

In this study, we compared two Chilean landraces of quinoa, one belonging to the *salares* ecotype (R49) and originating from the northern *altiplano* and another (Villarrica, VR) from a milder, rainier zone (coastal-lowlands ecotype), based on the assumption that the former may be better adapted to salinity. A similar rationale has been used in several other studies aimed at comparing quinoa genotypes (Shabala et al., 2013; Bonales-Alatorre et al., 2013; Bendevis et al., 2014; Ruiz et al., 2016a). Plant responses to salt stress and drought share a number of features and metabolic and signaling pathways (Golldack et al., 2014). Thus, based on genome-wide transcriptomic analyses conducted in quinoa under drought stress (Morales et al., 2017; Raney et al., 2014) and on our earlier data regarding growth, biochemical, and molecular responses to salinity in Chilean landraces (Orsini et al., 2011; Ruiz-Carrasco et al., 2011; Ruiz et al., 2016a), time-course changes in the relative transcript abundance of the following genes were investigated from 0.5 to 120 h after transfer to saline medium: (i) genes involved in growth; (ii) ion homeostasis genes; (iii) PA biosynthetic, and oxidative genes, and (iv) a proline biosynthetic gene. Given the paucity of information on the salt-induced ABA response in quinoa, we also investigated ABA biosynthetic, perception, and conjugate cleavage genes. Transcript levels of ABA-responsive and other stress-related transcription factors, were also evaluated. To determine how the predicted functions of ABA and PAs in the salt response corresponded to the temporal kinetics of genes encoding for biosynthesis and degradation enzymes, we quantified the levels of these growth regulators.