ATIVAÇÃO DIFERENCIAL PRECOCE DE GENES DE TRANSPORTADORES DE NA^+ EM TECIDOS DE CULTIVARES DE *QUINOA* DIVERGENTES NA TOLERÂNCIA A SALINIDADE

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RESUMO: O estresse salino compromete severamente o crescimento e a produtividade das plantas, especialmente em regiões áridas e semiáridas. *Chenopodium quinoa*, uma halófita facultativa, apresenta diferenças genotípicas na tolerância ao sal, sendo um modelo valioso para estudar mecanismos de adaptação. Este estudo reanalisou dados transcriptômicos de raízes e caules de dois genótipos contrastantes, Q68 (tolerante) e Q30 (sensível), 30 minutos após exposição a 200 mM de NaCl. O foco foi em genes envolvidos na extrusão de Na^+ e homeostase iônica, incluindo NHX, SOS1, bombas de prótons (V-ATPase, PPase e H^+-ATPase de membrana plasmática) e genes relacionados à respiração mitocondrial (COX). Em Q68, o estresse salino inicial induziu forte regulação positiva de NHX2a/2b e isoformas de V-ATPase/PPase nas raízes, promovendo a compartimentalização vacuolar de Na^+, enquanto nos caules a resposta foi mais moderada. H^+-ATPases e SOS1 foram modestamente induzidos, apoiando a extrusão de Na^+. Em Q30, a ativação gênica foi mais fraca e descoordenada. Os resultados destacam as raízes como sítios-chave para respostas precoces ao sal e demonstram que a regulação gênica eficiente de Q68 sustenta sua superior tolerância inicial à salinidade.
PALAVRAS-CHAVE: transporte iônico, NHX, SOS1

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EARLY DIFFERENTIAL ACTIVATION OF NA^+ TRANSPORTERS GENES IN TISSUES OF *QUINOA* CULTIVARS WITH DIVERGENT SALINITY TOLERANCE

ABSTRACT: Salt stress severely hampers plant growth and productivity, particularly in arid and semi-arid regions. *Chenopodium quinoa*, a facultative halophyte, displays genotypic differences in salt tolerance, making it a valuable model for studying salinity adaptation mechanisms. This study reanalyzed transcriptomic data from shoots and roots of two *Quinoa* genotypes with contrasting salt tolerance, Q68 (salt-tolerant) and Q30 (salt-sensitive), 30 minutes after exposure to 200 mM NaCl. The focus was on genes associated with Na^+ extrusion and ion homeostasis, including NHX, SOS1, and proton pumps (V-ATPase, PPase, and plasma membrane H^+-ATPase), as well as genes linked to mitochondrial respiration (COX). In Q68, early salt stress triggered strong upregulation of NHX2a/2b and V-ATPase/PPase isoforms in roots, promoting vacuolar Na^+ sequestration, while shoots showed a more moderate response. Plasma membrane H^+-ATPases and SOS1 were modestly induced, supporting Na^+ extrusion. In contrast, Q30 exhibited weaker and less coordinated gene activation. These results highlight roots as key sites for early salt stress responses and demonstrate that Q68's rapid, targeted gene regulation underpins its superior early salt tolerance.

KEYWORDS: ion transport, NHX, SOS1

INTRODUCTION

Salinity is a critical abiotic constraint that occurs predominantly in arid and semi-arid regions of the world and is known to significantly reduce seed germination, plant development, and crop yield (Hanin et al., 2016; Julkowska et al., 2017). This stress disrupts cellular ion and water homeostasis, leading to ion imbalance, as well as osmotic and oxidative stresses, which can ultimately culminate in cell death (Zhu, 2001; Lv et al., 2019).

To cope with salt stress, plants have evolved a range of adaptive mechanisms that integrate salinity sensing and signaling pathways with internal developmental cues. This coordination enables the rapid restoration of ionic balance, osmotic stability, and control of reactive oxygen species (ROS), thereby fine-tuning the balance between growth and stress adaptation (Shi & Gu, 2020).

Among the various strategies to preserve cellular homeostasis under salt stress, plants employ complementary mechanisms to remove excess Na^+ from the cytoplasm. One such

mechanism involves Na^+/H^+ antiporters, such as the SOS1 protein (Shi et al., 2000), which actively extrudes Na^+ across the plasma membrane and contributes to long-distance Na^+ transport between roots and shoots. Another strategy is the sequestration of Na^+ into vacuoles, where the ion is safely stored away from the cytoplasm via NHX antiporters (Apse et al., 1999). Both NHX and SOS1 are energized by the electrochemical gradient generated by vacuolar ATPase (V-ATPase) and pyrophosphatase (PPase) in the vacuole, or by plasma membrane H^+-ATPases (PHA), respectively (Gaxiola et al., 2007; Martínez-Atienza et al., 2007).

In addition, plants can compartmentalize Na^+ in specific tissues such as roots and stems, thereby preventing its accumulation in more sensitive photosynthetic tissues in the shoots (Munns & Tester, 2008). Some plant species, particularly halophytes, possess specialized structures, such as salt glands and epidermal bladder cells (EBCs) that actively excrete Na^+ to the external environment (Flowers & Colmer, 2008).

Halophytes are plants with well-characterized adaptations that allow them to survive in environments with high salt concentrations. In this context, *Quinoa* (*Chenopodium quinoa*) is considered a facultative halophyte, displaying genotypic variation in salt stress tolerance (Adolf et al., 2013). Therefore, *Quinoa* serves as a promising plant model for investigating the diversity of salt tolerance mechanisms.

In a previous study, Vita et al. (2021) performed early (30-minute) transcriptome analyses of shoots and roots in two *Quinoa* genotypes with contrasting salt tolerance and EBC density: Q30 (salt-sensitive, high EBC density) and Q68 (salt-tolerant, low EBC density). The authors reported that the main transcriptomic changes involved hormone signaling (ABA and ethylene) and stress-responsive genes, which were predominantly upregulated in the sensitive genotype. However, the study did not detect or emphasize significant involvement of Na^+ extrusion-related systems. Thus, in the present work, we reanalyzed the publicly available transcriptomic dataset (GenBank) from Vita et al. (2021) to investigate the expression profiles of gene families involved in cytosolic Na^+ extrusion, including NHX, V-ATPase subunit A (catalytic), PPase, PHA, and SOS1. We also evaluated genes from cytochrome oxidase (COX) to monitor the mitochondrial respiration and ATP supply. Our goal was to identify specific gene members potentially contributing to salt tolerance in Quinoa.

MATERIAL AND METHODS

Experimental Conditions of *Quinoa* Transcriptomes Used in Gene Expression Analyses

Vita et al. (2021) describe accordingly these experimental conditions. Seeds of Q30 and Q68 genotypes were surface-sterilized, germinated on moist filter paper at 25 °C for 48 h, and then transferred to aerated ¼-strength Hoagland solution. After 24 h of acclimation in controlled conditions (23/20 °C, 12 h photoperiod, 200 μmol m^{-2} s^{-1}), seedlings were treated with 200 mM NaCl or maintained in control solution for 30 minutes prior to harvest for transcriptomic analysis. The data were available in 3 biological replicates.

Search of genes/cDNAs from NHX, V-ATPase subunit A (catalytic), PPase, PHA, SOS1 and COX in Quinoa

Homologous proteins from *Arabidopsis* were used in tBLASTn searches to retrieve genes (Ref Seq representative genome) and cDNAs (Refseq RNA) from GenBank databases. After, the corresponding cDNAs were classified by phylogenetic analyses using the MEGA software (Kumar et al., 2018) and the homologous *Arabidopsis* cDNAs as reference.

Gene Expression Analyses in Transcriptome Data of *Quinoa* Genotypes

The analysis of gene expression based on transcriptomic raw data from Vita et al. (2021) was performed using three biological replicates per sample and followed a three-step approach. First, RNA-seq reads were aligned to the target cDNA sequences using the Magic-BLAST tool (Boratyn et al., 2019). Next, the number of aligned reads was quantified using HTSeq (Anders et al., 2015). Finally, read counts were normalized across all samples. For the alignment step, target cDNAs served as reference sequences for read mapping. Expression levels were then normalized using the RPKM (Reads Per Kilobase per Million reads) method [Mortazavi et al., 2008], applying the formula: RPKM = (number of mapped reads × 10^9) / (total reads in the dataset × gene length in nucleotides). In the case of multigene families, expression values were reported as the sum of RPKM values from all family members, denoted as “total genes.”

Statistical Analysis

The gene expression data was expressed as the mean ± standard deviation (SD) of three biological replicates. The averages obtained from control and salt stress treatments were compared for each gene separately using Student’s t-test at a 5% significance level in GraphPad Prism 5.0 software.

RESULTS AND DISCUSSION

Genes of NHX, SOS1, V-ATPase subunit A (catalytic), PPase, PHA and COX in Quinoa

Genomic DNA/RNA and phylogenetic analyses revealed that all studied genes belong to multigene families, comprising 8 NHX genes (NHX1a, 1b, 2a, 2b, 4a, 4b, 5a, and 5b), 2 SOS1 genes (SOS1a and SOS1b), 2 V-ATPase subunit A genes (A1 and A2), 6 V-PPase genes (PPase1a, 1b, 1c, 1d, 2a, and 2b), 19 PHA genes (PHA1a, 1b, 2a, 2b, 3a, 3b, 7a, 7b, 8a, 8b, 9a, 9b, 10a, 10b, 11a, 11b, 11c, 11d, and 11e), and 16 COX genes.

Regarding Na^+ extrusion genes (NHX and SOS1), our data match the same 10 genes recently reported by Santhoshi et al. (2025). According to these authors, the set includes six vacuolar proteins (NHX1a, 1b, 2a, 2b, 4a, and 4b), two endosomal proteins (NHX5a and 5b), and two plasma membrane proteins (SOS1a and 1b). Thus, it can be inferred that all NHX and SOS1 genes involved in Na^+ extrusion in *Quinoa* cells were addressed in this study.

However, for V-ATPase subunit A, V-PPase, and PHA genes, no previous reports were found detailing the number of genes and the characterization of these families, despite the *Quinoa* reference genome having been published in 2017 (Jarvis et al., 2017). For PHA, the 19 genes identified in *Quinoa* databases clustered into six PHA types (1–3, 7, 8, 9, 10, and 11) based on phylogenetic analysis with *Arabidopsis* homologs (Figure 1a). This distribution is similar to the five groups previously suggested for plant PHA by Arango et al. (2003), differing only in that those authors grouped types 8 and 9 together. Based on the phylogenetic tree (Figure 1a), *Quinoa* possesses orthologous members corresponding to all 12 *Arabidopsis* PHA genes previously characterized (Palmgren & Harper, 1999; Palmgren, 2001).

For V-PPase, comparative analyses between *Quinoa* and *Arabidopsis* suggest that gene duplication events occurred in *Quinoa* V-PPase1 (PPase1a–1d), whereas a similar number of genes was observed for V-PPase2 (Figure 1b). Duplicated genes were also observed for the catalytic V-ATPase subunit A (designated as V-ATPase-A1 and A2).

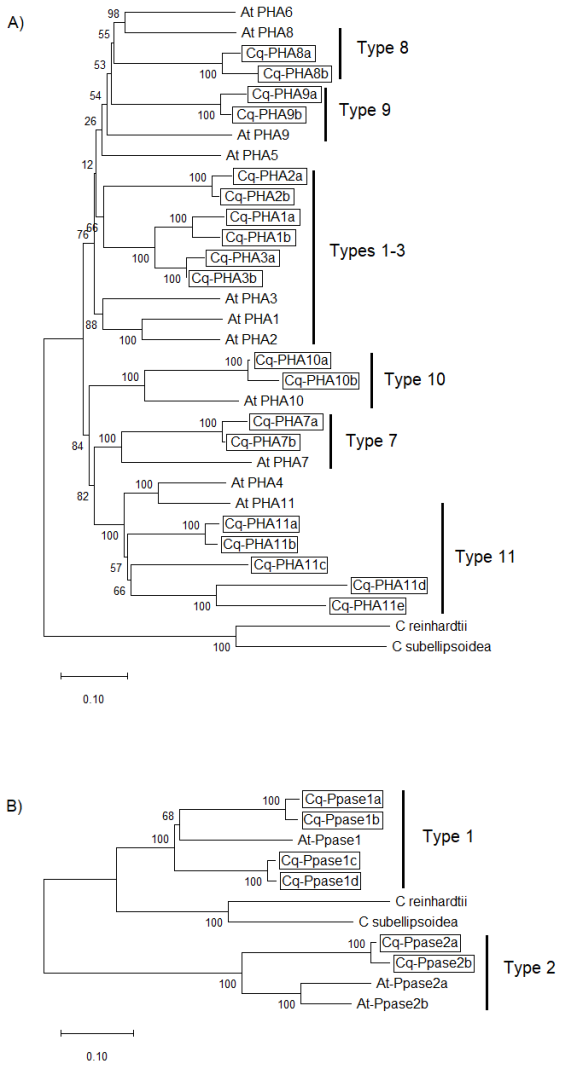


Figure 1. Phylogenetic relationships of (A) PHA and (B) PPase cDNAs from *Chenopodium quinoa* (Cq), *Arabidopsis* thaliana (At), *Chlamydomonas reinhardtii* (*C. reinhardtii*), and *Chlorella subellipsoidea* (*C. subellipsoidea*). Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method from multiple sequence alignments of full-length cDNA sequences. Bootstrap values (1,000 replicates) are indicated at the corresponding nodes. The classification of *C. quinoa* sequences into types follows the grouping defined by A. *thaliana* homologs, which were used as reference sequences. The two green algal sequences served as outgroup to root the trees. The scale bar represents the number of nucleotide substitutions per site.

The higher number of genes in *Quinoa* observed here consists of closely related gene pairs (approximately 95% identity), designated as “a” and “b” in Figure 1. These findings likely reflect intrinsic features of Quinoa, which arose from the hybridization of two ancestral diploid genome species (Kolano et al., 2016; Jarvis et al., 2017).

Total Gene Expression in Shoots and Roots under Short-Term Salinity Stress

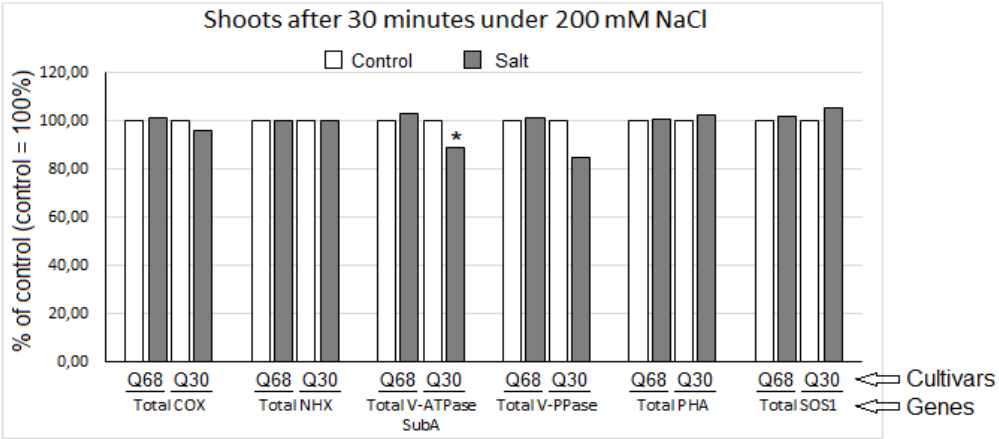
The total gene expression comprised by the sum of the transcript levels of all gene members from each family allowed us to take a general view on their salt stress response.

The early transcriptional responses to 200 mM NaCl in *Quinoa* reveal clear differences between the salt-tolerant (Q68) and salt-sensitive (Q30) cultivars, emphasizing organ-specific strategies (Figure 2). In shoots, Q68 maintained stable expression of most genes, suggesting effective maintenance of ionic homeostasis, whereas Q30 showed downregulation of vacuolar proton pumps (V-ATPase and V-PPase) (Figure 2a), indicating early impairment of Na^+ compartmentalization, consistent with reports linking reduced vacuolar H^+-pump activity to salt sensitivity (Apse et al., 1999; Zhang et al., 2015). Slight upregulation of SOS1 in both cultivars may reflect initial Na^+ extrusion, though activation likely occurs later (Shi et al., 2000).

Roots displayed more prominent responses (Figure 2b). In Q68, NHX and V-ATPase Subunit A were significantly upregulated, with moderate induction of V-PPase, indicating early activation of vacuolar Na^+ sequestration and pH regulation. Increased COX transcript levels further suggest enhanced ATP production to support active ion transport, consistent with mechanisms described for tolerant genotypes (Bassil et al., 2012; Yang et al., 2014). Q30 roots showed limited induction of these transporters, reflecting reduced capacity for vacuolar sequestration. Both cultivars exhibited trends toward increased PHA and SOS1 transcripts, suggesting early general mechanisms for Na^+ extrusion (Zhu, 2003).

Overall, these results support the model that early root-specific activation of vacuolar ion transport and energy metabolism underlies salt tolerance in Quinoa, with spatial regulation between roots and shoots critical for initial stress adaptation (Munns & Tester, 2008).

A)



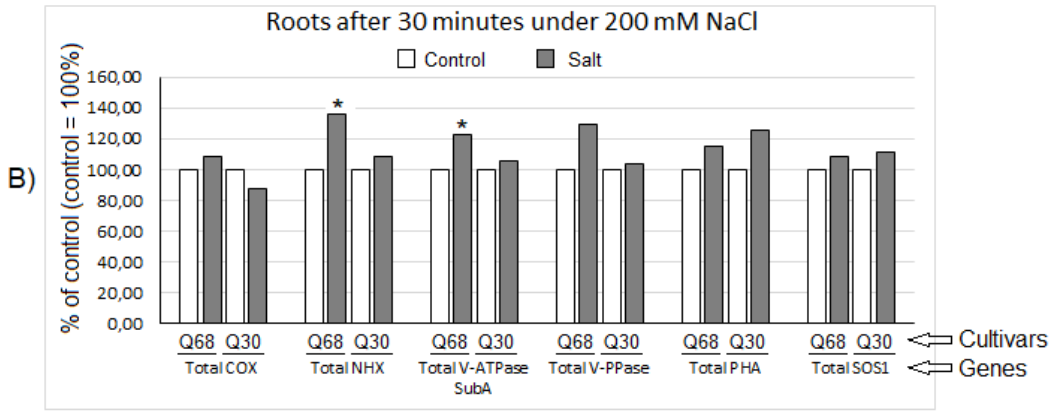


Figure 2. Relative transcript levels of genes related to ion transport and energy metabolism in *Quinoa* after 30 minutes of salt treatment (200 mM NaCl). Shoots (a) and roots (b) of two *Quinoa* cultivars—Q68 (salt-tolerant) and Q30 (salt-sensitive). Bars represent the total transcript accumulation (percentage) of the genes COX, NHX, V-ATPase subunit A, V-PPase, Plasma membrane H^+-ATPase (PHA), and SOS1 under control (white bars) and salt (gray bars) conditions. Data are shown as percentage relative to control levels for each gene and cultivar. Asterisks indicate significant values at $p < 0.05$.

Main Specific Genes Involved in Na^+ Extrusion under Short-Term Salinity Stress

Figure 3 shows the log2 fold change (heatmap) of selected genes involved in Na^+ extrusion in the roots of the two *C. quinoa* genotypes.

In Q68, early salt stress triggered a marked upregulation of NHX2a and NHX2b, gene members known to play central roles in vacuolar Na^+ storage and K^+ homeostasis (Bassil et al., 2011), while other NHX members (NHX1a/1b, NHX5a/5b) remained largely unchanged, suggesting a targeted rather than generalized activation. This response was accompanied by higher expression of V-ATPase SubA1 and SubA2, supported by mild induction of multiple V-PPase isoforms (Ppase1a, 1c, 1d, 2a, 2b), a combination that enhances the proton motive force needed for NHX activity (Silva & Gerós, 2009; Gaxiola et al., 2001).

At the plasma membrane, Q68 showed moderate increases in PHA3b, PHA2a, and PHA2b, gene members associated with energizing Na^+/H^+ exchange, whereas Q30 presented slightly higher levels of PHA3a. Although SOS1a and SOS1b were marginally more expressed in Q30, differences were small and likely less relevant than the upstream capacity to generate the driving force for Na^+ extrusion (Qiu et al., 2002).

The overall pattern in Q68 thus reflects a coordinated adjustment of specific pump–transporter pairs NHX2–V-ATPase/V-PPase and PHA2/3–SOS1 that is less evident in Q30, potentially explaining the superior early tolerance of the former.

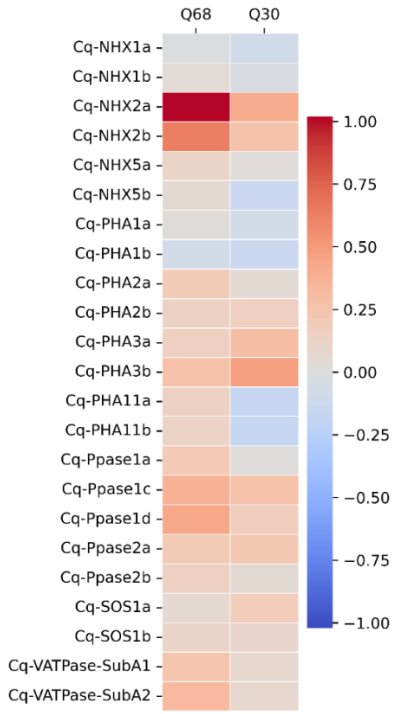


Figure 3. Heatmap of Log2 fold change from the main specific genes involved in Na^+ extrusion in roots from *Quinoa* after 30 minutes of salt treatment (200 mM NaCl). The scale represents Log2 fold change variation of 1 to -1.

CONCLUSION

In summary, under short-term salinity stress, the salt-tolerant *Quinoa* genotype Q68 exhibited a coordinated upregulation of specific genes crucial for Na^+ extrusion and compartmentalization. NHX2a and NHX2b were strongly induced, supporting vacuolar Na^+ sequestration, while V-ATPase and V-PPase specific genes linked to enhanced proton motive force necessary for antiporter activity. At the plasma membrane, moderate increases in PHA2/3 isoforms likely energized Na^+/H^+ exchange, whereas SOS1 expression showed minimal differences between genotypes. In contrast, the salt-sensitive Q30 displayed weaker or less coordinated gene activation. These results indicate that Q68's early salinity tolerance relies on targeted, efficient regulation of pump–transporter pairs, ensuring rapid Na^+ compartmentalization and extrusion, a mechanism less pronounced in Q30.

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