Characterization of AQP genes in *Zea mays* under saline stress: a genomic and functional approach

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ABSTRACT: Aquaporin (AQP) is an integral membrane protein responsible for water transport through cell membranes in all organisms. In plants such as maize (*Zea mays*), AQPs play a key role in maintaining cellular homeostasis and controlling turgor pressure, which are critical under saline stress conditions. In this study, we evaluated the expression of 41 AQP-encoding genes in leaves and roots of *Zea mays* subjected to saline stress over a 168-hour exposure period. We identified 26 genes exhibiting significant expression levels (RPKM > 1) during the treatment. AQP gene expression was higher in roots, with peaks observed at 24, 48, 120 and 168 hours, whereas in leaves expression increased at 48, 72, and 168 hours. These results suggest the activation of distinct molecular mechanisms in different tissues, contributing to maize's adaptive response to saline stress.

KEYWORDS: gene expression, salt stress, aquaporin

Caracterização dos genes AQP em *Zea mays* sob estresse salino: abordagem genômica e funcional

RESUMO: A aquaporina (AQP) é uma proteína integral de membrana que atua no transporte de água através das membranas celulares de todos os organismos. Em plantas como o milho (*Zea mays*), as AQPs desempenham papel fundamental na manutenção da homeostase celular e no controle da turgescência, aspectos cruciais em condições de estresse salino. Neste estudo, avaliamos a expressão de 41 genes codificadores de AQP em folhas e raízes de *Zea mays* submetidas ao estresse salino ao longo de 168 horas de exposição. Um total de 26 genes de AQP apresentou expressão significativa (RPKM > 1) durante o tratamento. A expressão gênica

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de AQP foi maior nas raízes, com picos em 24, 48, 120, e 168 horas. Nas folhas, a expressão aumentou em 48, 72, e 168 horas. Tais resultados sugerem a ativação de mecanismos moleculares distintos em tecidos diferentes, contribuindo para a resposta adaptativa do milho ao estresse salino.

PALAVRAS-CHAVE: expressão gênica, estresse salino, aquaporina

INTRODUCTION

Water scarcity remains one of the primary constraints on plant development and agricultural productivity, particularly under conditions of salinity stress. Saline environments, prevalent in irrigated and arid regions, significantly reduce soil water potential, thereby limiting the capacity of roots to absorb water efficiently. This reduction in water uptake leads to osmotic stress, accompanied by ionic toxicity and nutrient imbalances, which together disrupt key physiological processes such as germination, root elongation, and shoot development (Hasanuzzaman & Fujita, 2022; Zhao et al., 2021). The multifaceted nature of salt stress requires a comprehensive understanding of the molecular and physiological mechanisms that enable plants to maintain homeostasis and survive under such adverse conditions. Enhancing this knowledge is fundamental for developing stress-resilient crop varieties and promoting sustainable agricultural practices.

In order to cope with such conditions, plants activate a range of molecular and physiological mechanisms, including aquaporins (AQPs) – integral membrane proteins responsible for the selective transport of water and small solutes, which are essential for maintaining cellular homeostasis (Maurel et al., 2015; Finch-Savage & Bassel, 2016; Chaumont & Tyerman, 2017). A genome-wide analysis of AQP genes in maize has identified 41 members, distributed across different subcellular compartments and exhibiting distinct spatial and temporal expression profiles (Su et al., 2022). Notably, this characterization was restricted to developmental stages such as seed germination and early seedling growth under standard, non-stress conditions. The number of AQP genes identified varies significantly among plant species, a reflection of both genomic diversity and species-specific physiological demands. While *Arabidopsis thaliana* contains approximately 35 AQP genes, *Oryza sativa* has about 33, and other crops like tomato, cotton, and soybean possess between 34 and 66 (Maurel et al., 2015; Bansal & Sankararamakrishnan, 2007).

Maize (*Zea mays*) stands out as the world's most cultivated cereal, contributing over 30% of caloric intake for approximately 4.5 billion people in 94 developing nations, with global production exceeding one billion tonnes annually (Farooq et al., 2015; Erenstein et al., 2022). However, despite its agricultural prominence, maize exhibits moderate sensitivity to saline environments, which can negatively influence key physiological functions such as seed germination, nutrient absorption, and photosynthetic efficiency – ultimately impairing yield. Given the complexity of plant responses to salinity, investigating the expression and regulation of AQP genes under salt stress is crucial to advancing our understanding of adaptive mechanisms. Such knowledge may also facilitate the development of improved cultivars through breeding strategies aimed at enhancing stress resilience.

In maize, AQPs are grouped into five distinct subfamilies according to their subcellular localization and functional roles. Each subfamily corresponds to a specific membrane system within the cell: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and X intrinsic proteins (XIPs). This classification reflects the diversity of AQP-mediated transport pathways across different cellular compartments (Danielson & Johanson, 2008; Maurel et al., 2015).

Numerous studies have shown that aquaporin (AQP) gene expression is dynamically regulated in response to salt stress across diverse plant species, underscoring their functional relevance in abiotic stress tolerance. In *Arabidopsis thaliana*, for instance, exposure to NaCl triggers a rapid transcriptional downregulation of various AQPs, particularly members of the PIP and TIP families, with changes detectable as early as 2–4 hours post-treatment. This decline in gene expression is accompanied by reduced root hydraulic conductivity, indicating compromised water transport capacity under saline conditions (Boursiac et al., 2005). In contrast, salt-induced upregulation of specific AQPs has been observed in other species. In *Musa acuminata*, *MaPIP1*;1 expression increased under salt stress, and its heterologous expression in Arabidopsis conferred enhanced tolerance by minimizing membrane damage and promoting osmotic balance (Xu et al., 2014). Similarly, in the halophytic species *Sesuvium portulacastrum, SpAQP1* (a PIP2-type aquaporin) was highly induced by salinity, and its overexpression in tobacco improved germination, root elongation, and antioxidant enzyme activity under stress (Chang et al., 2015). Collectively, these studies highlight the evolutionarily conserved yet context-dependent roles of AQPs in mediating plant responses to saline environments.

In maize, certain aquaporin genes – such as ZmPIP1-1, ZmPIP1-5, and ZmPIP2-4 – exhibit transient upregulation in the root system during early exposure to moderate salt stress

(100 mM NaCl), particularly in the outer root tissues, after 2 hours of treatment. However, under more severe saline conditions (200 mM NaCl), the expression of most *ZmPIP* and *ZmTIP* genes is markedly suppressed after 24 hours, demonstrating that AQP gene regulation is not only responsive to salinity but also modulated according to the intensity and duration of the stress (Zhu et al., 2005).

Although some efforts have examined aquaporin regulation in maize under salinity, they remain limited in scope. For example, Zhu et al. (2005) reported early AQP upregulation in outer root tissues following exposure to moderate NaCl concentrations, yet their analysis did not include leaves. Transcriptome-wide studies using time-course clustering have revealed dynamic gene expression patterns in maize seedlings under salt stress, but these did not focus on AQP genes nor provide tissue-specific resolution across a detailed time scale (Zhang et al., 2023). Consequently, a comprehensive, multi-tissue, time-course examination of AQP expression distinguishing rapid (within first 24 h) versus delayed (24–168 h) responses in roots and leaves is currently lacking.

This study aims to characterize the genomic profile and expression dynamics of AQP genes in maize roots and leaves exposed to salt stress over a 168-hour period, providing key insights into the temporal and tissue-specific responses that could aid in developing salt-tolerant cultivars. By integrating gene family analysis with tissue-specific and time-course expression profiling, we investigate the differential regulation of AQP genes in response to salinity. Particular emphasis is placed on distinguishing early-responsive genes (within the first 24 hours) from late-responsive ones (24 to 168 hours), in order to elucidate both immediate and sustained transcriptional responses associated with salt stress adaptation. These findings may contribute to the identification of key AQP candidates for improving maize salinity tolerance.

MATERIALS AND METHODS

Gene/cDNA/protein sequences of AQP genes were retrieved from NCBI databases. Expression data were obtained from BioProject PRJNA670840, which includes transcriptomic data from the maize cultivar Jing724 exposed to 100 mM NaCl treatment for 168 hours (Luo et al., 2021). AQP gene expression analysis in the transcriptomic data was conducted in three steps: (1) read mapping using the Magic-BLAST software (Boratyn et al., 2019); (2) quantification of mapped reads using HTSeq (Anders & Huber, 2015); and (3) normalization of read counts across all samples. After read quantification, normalization between samples was

performed using the RPKM (Reads Per Kilobase of transcript per Million mapped reads) method (Mortazavi et al., 2008), according to the following equation: RPKM = (number of mapped reads × 10^9) / (number of sequences in each dataset × number of nucleotides in each cDNA). Log_2 Fold Change was also calculated to compare gene expression between the treatment and control groups for the studied genes.

RESULTS AND DISCUSSION

AQPs play a crucial role in plant adaptation to salt stress, a finding further supported by the expression patterns of their corresponding genes under stress conditions. The sum of normalized counts (Figure 1) revealed a higher overall expression of AQPs in roots compared to leaves, with significant changes observed at 24, 48, 120, and 168 hours after NaCl exposure. In contrast, in leaves, elevated expression was primarily detected at 48, 72, and 168 hours. These results underscore the tissue-specific nature of AQP regulation under salt stress, consistent with previous studies that have shown more rapid and pronounced AQP expression in roots early in response to salinity, whereas leaves exhibit a delayed response (Zhu et al., 2002).

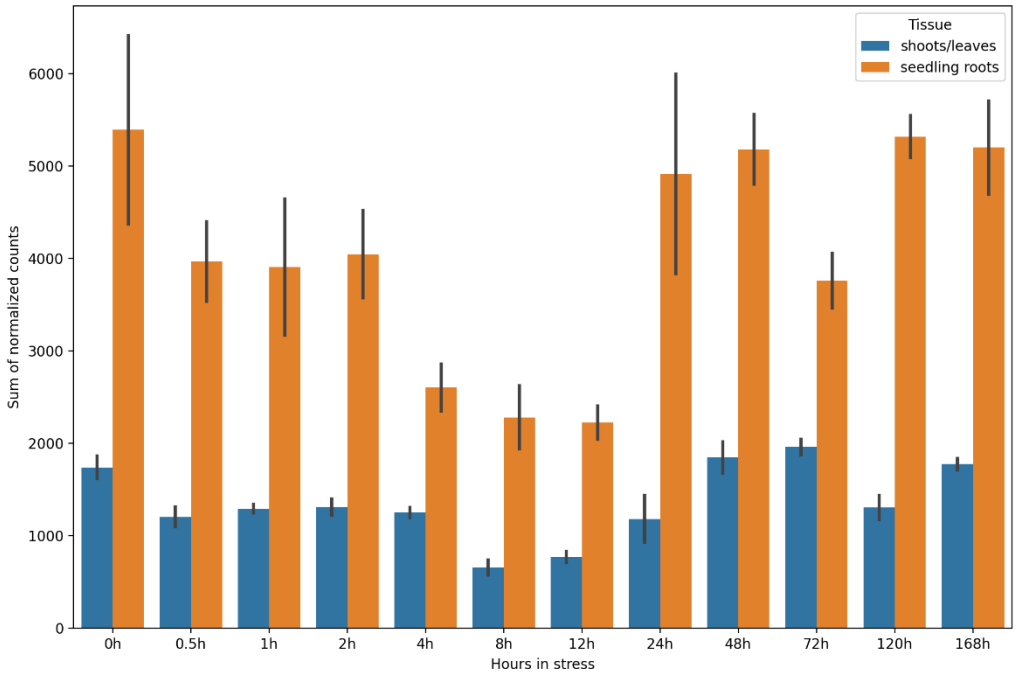

Figure 1. Total AQP gene expression in maize leaves and roots at different time points under salt stress.

We identified 26 AQP genes with significant expression levels (RPKM > 1) in this experiment. Table 1 provides an overview of the genes that were upregulated and downregulated in roots and leaves ($|\log2FC| \geq 1$). Among the 26 differentially expressed genes, 21 in roots showed a rapid response (within 24 hours), including 4 NIPs, 11 PIPs, and 6 TIPs.

Table 1. Quantification of upregulated and downregulated genes in leaves and roots throughout salt stress whole exposure.

Class	Tissue			
	Root		Leaf	
	Upregulated	Downregulated	Upregulated	Downregulated
NIP	2	2	0	5
PIP	0	12	2	7
SIP	0	0	0	1
TIP	4	3	2	3

The upregulated genes in roots during the first 24 hours of salt stress exposure included *ZmNIP1-1*, *ZmNIP2-3*, *ZmTIP3-3n*, *ZmTIP4-1*, and *ZmTIP4-2* (Figure 2). Their expression patterns throughout the entire salt exposure period are shown in Figure 2. ZmNIP1-1 exhibits a peak in expression between 8 and 24 hours, followed by a sharp decline. In contrast, *ZmTIP4-1* and *ZmTIP4-2* showed higher expression at the 8-hour mark, indicating a more transient response during the initial stages of salt stress.

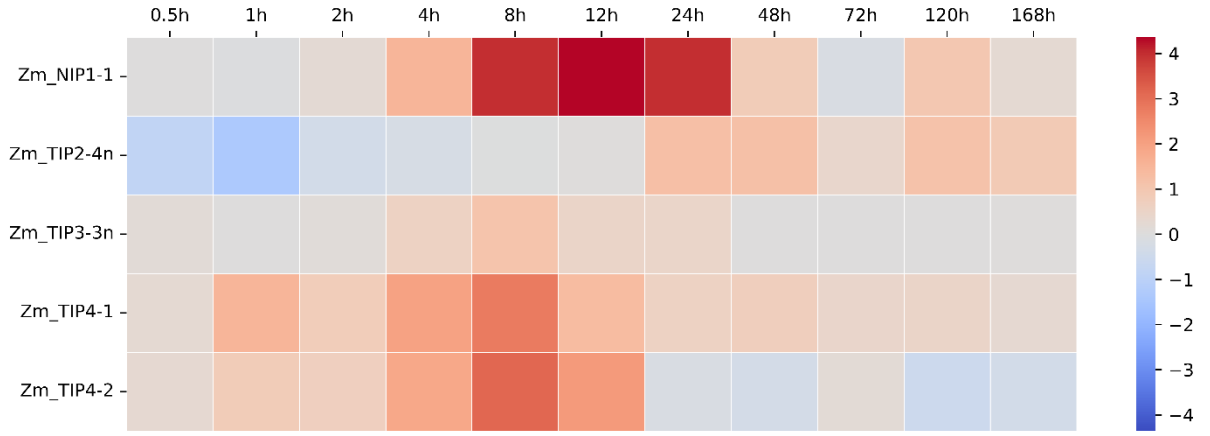

Figure 2. Heatmap of Log2 Fold Change comparing gene expression under salt stress to control in roots.

The upregulation of specific AQP genes in response to salt stress observed in this study is consistent with findings from other plant species. For instance, *ZmTIP4-1* and *ZmTIP4-2*,

which were upregulated both in roots and leaves in our experiment, have been shown to play significant roles in mediating water transport under saline conditions in maize (Zhu et al., 2002).

During the first 24 hours of salt stress exposure, the genes *ZmPIP1-3* and *ZmPIP2-2* were the only ones upregulated in leaves. As shown in Figure 3, both genes exhibited a rapid increase in expression within the first 4 hours of exposure, after which their expression declined significantly. This early peak in expression suggests a transient activation of these aquaporins, likely contributing to the initial response to salinity. The subsequent decline in expression may reflect a shift in the plant's regulatory mechanisms as it adapts to prolonged salinity stress. Zhu et al. (2005) had previously observed repression of multiple *ZmPIP* and *ZmTIP* genes under salt stress (200 nM NaCl) after 24 hours.

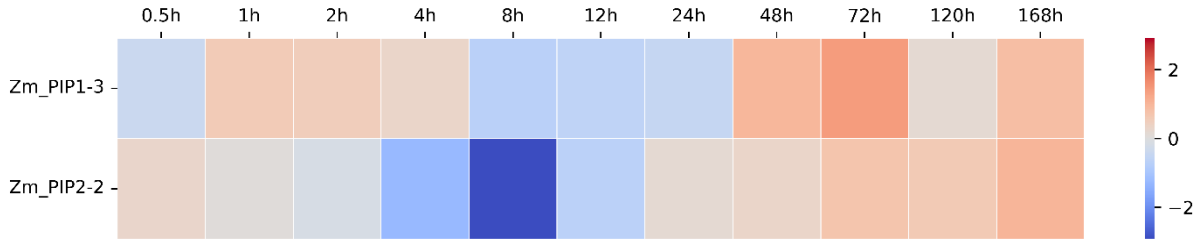

Figure 3. Heatmap of Log2 Fold Change comparing gene expression under salt stress to control in leaves.

CONCLUSIONS

The results indicate that the expression of AQP gene family members is differentially regulated between roots and leaves of Zea mays under salt stress, both in intensity and timing. The higher overall AQP expression observed in roots suggests a more active response in this tissue, possibly related to water uptake and flow regulation under salinity. The upregulation of *ZmNIP2-4n*, *ZmNIP2-3*, *ZmTIP2-4n*, *ZmTIP3-3n*, *ZmTIP4-1*, *e ZmTIP4-2* in roots reinforces the role of tonoplast and nodulin-26-like AQPs in osmotic regulation and solute compartmentalization. In leaves, the activation of *ZmPIP1-3*, *ZmPIP2-2*, *ZmTIP4-1*, *e ZmTIP4-2* highlights the contribution of plasma membrane and tonoplast aquaporins to turgor maintenance and water balance. These expression patterns, together with the subcellular distribution of AQP subgroups, suggest that the salt stress response involves coordinated, tissue-specific mechanisms mediated by different AQP classes. These genes represent promising targets for genetic improvement strategies using biotechnological tools aimed at enhancing salinity tolerance.

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