

## Transcriptomic analysis of the aquaporin gene family in salt-contrasting maize cultivars reveals candidate genes for stress resilience

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**ABSTRACT:** Aquaporins (AQPs) are membrane proteins that facilitate water transport and play a key role in plant adaptation to salinity by maintaining turgor and cellular homeostasis. In this study, we analyzed the expression of 41 AQP genes in roots of two *Zea mays* inbred lines with contrasting salt tolerance – P138 (salt-sensitive) and 8723 (salt-tolerant) – after exposure to 180 mM NaCl for approximately 10 days. A total of nine AQP genes were significantly upregulated under salt stress, particularly in the tolerant genotype. Notably, specific aquaporins of the subfamilies NIP (nodulin-26-like protein) and PIP (plasma membrane intrinsic protein) were differentially expressed between genotypes, suggesting their involvement in osmotic adjustment. These genes may serve as molecular markers or targets for breeding salt-tolerant maize varieties.

**KEYWORDS:** aquaporins, salt stress, gene expression, maize, transcriptome

**Análise transcriptômica das aquaporinas em dois cultivares de milho com tolerância  
contrastante à salinidade revela genes candidatos à resiliência ao estresse**

**RESUMO:** As aquaporinas (AQPs) são proteínas de membrana que facilitam o transporte de água e desempenham um papel fundamental na adaptação das plantas à salinidade, contribuindo para a manutenção do turgor e da homeostase celular. Neste estudo, analisamos a expressão de 41 genes codificadores de AQPs em raízes de duas linhagens endogâmicas de *Zea mays* com contrastante tolerância ao sal – P138 (sensível) e 8723 (tolerante) – após exposição a 180 mM de NaCl por aproximadamente 10 dias. Um total de nove genes apresentou regulação positiva sob estresse salino, especialmente na linhagem tolerante. Notadamente, aquaporinas das subfamílias NIP (proteína tipo nodulina-26) e PIP (proteína intrínseca de membrana plasmática) foram diferencialmente expressas entre os genótipos, sugerindo seu envolvimento na regulação osmótica. Esses genes podem atuar como marcadores moleculares ou alvos para programas de melhoramento genético visando à tolerância à salinidade em milho.

**PALAVRAS-CHAVE:** aquaporinas, estresse salino, expressão gênica, milho, transcriptoma

## INTRODUCTION:

Water availability is one of the most significant limiting factors for plant growth and productivity. This limitation becomes even more critical under salt stress, a common abiotic stress in many agricultural regions, which reduces the ability of plants to absorb water from the soil. High salinity leads to a decrease in the soil's water potential, making it more difficult for plant roots to take up water efficiently. As a result, salt stress can severely affect various aspects of plant development, including seed germination, root and shoot growth, and overall physiological performance (Hasanuzzaman & Fujita, 2022). Salt stress not only creates osmotic stress—making water less available to plants—but also leads to ion toxicity and nutrient imbalances, negatively impacting germination, growth, and yield (Zhao et al., 2021). In this context, understanding how plants respond at the molecular and physiological levels is essential to improving crop resilience and sustainability in agriculture.

To survive and adapt to such adverse conditions, plants have evolved a wide range of molecular and physiological responses. Among these, the regulation of aquaporins (AQPs) plays a central role. AQPs are a family of membrane-intrinsic proteins that facilitate the transport of water and, in some cases, small solutes across cellular membranes. These proteins are essential for maintaining water balance within plant cells and tissues under both normal and stress conditions and are actively involved in the plant's ability to respond to environmental stresses. Under salt stress, AQP expression and activity can be modified to help plants control water movement, avoid dehydration, and preserve cellular homeostasis (Maurel et al., 2015;

Finch-Savage & Bassel, 2016; Chaumont & Tyerman, 2017). The AQP gene family in *Zea mays* is classified into five subfamilies based on subcellular localization and functional characteristics. This classification reflects the typical cellular compartment in which each protein operates: 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) (Maurel et al., 2015; Danielson & Johanson, 2008).

In maize (*Zea mays*), a crop of major economic and agricultural relevance worldwide, salinity represents a serious threat to productivity, particularly in regions with irrigated or marginal soils (Farooq et al., 2015; Erenstein et al., 2022). Therefore, identifying and characterizing AQP genes responsive to salt stress is not only scientifically relevant but also of practical importance. Insights into how these genes are regulated can help clarify the mechanisms of stress adaptation that enable this species to tolerate salinity. Moreover, identifying specific AQPs that are responsive to salinity may support future breeding efforts aimed at improving maize performance under challenging environmental conditions.

Previous studies of comprehensive genomic characterization of AQPs performed in maize identified 41 genes presenting varying subcellular locations and spatiotemporal expression patterns (Su et al., 2022). However, their expression analysis was limited to seed germination and early seedling development under non-stress conditions, focusing solely on developmental regulation. The number of AQP genes varies considerably among plant species, reflecting both genome complexity and adaptive requirements. For example, *Arabidopsis thaliana* possesses approximately 35 aquaporin genes, rice (*Oryza sativa*) around 33, while other species such as tomato, cotton, and soybean contain between 34 and 66 AQP genes (Maurel et al., 2015; Bansal & Sankararamakrishnan, 2007)

Under salt stress conditions, several studies have demonstrated that aquaporin expression is modulated in a wide range of plant species, reinforcing their crucial role in stress adaptation. In *Arabidopsis thaliana*, it was reported that salt stress rapidly downregulates the expression of several aquaporins, particularly those from the PIP and TIP subfamilies. This transcriptional repression was observed as early as 2 to 4 hours after NaCl exposure. A concurrent decrease in root hydraulic conductivity was also noted, suggesting a direct impact on water uptake under saline conditions. (Boursiac et al., 2005). In *Musa acuminata* (banana), the gene MaPIP1;1 was found to be upregulated by salt treatment, and its overexpression in *Arabidopsis* enhanced salt tolerance by reducing membrane damage and improving osmotic adjustment (Xu et al., 2014). Similarly, in the halophyte *Sesuvium portulacastrum*, the aquaporin SpAQP1 (a PIP2-type gene) was strongly induced by NaCl, and transgenic tobacco

plants expressing this gene exhibited improved germination, root growth, and antioxidant enzyme activity under saline conditions (Chang et al., 2015). These findings across species support the conserved yet flexible role of aquaporins in plant salt stress responses.

Studies in maize have shown that specific AQP genes such as ZmPIP1-1, ZmPIP1-5, and ZmPIP2-4 are transiently upregulated in maize roots during short-term exposure to moderate salinity (100 mM NaCl), especially in the outer root layers (Zhu et al., 2005). At higher salinity levels (200 mM NaCl), most ZmPIP and ZmTIP genes are significantly repressed, indicating that AQP expression is not only stress-inducible but also highly dependent on stress intensity and duration. Despite these findings, studies on contrasting maize genotypes under salinity are still limited, although physiological evidence suggests significant variability in salt tolerance among cultivars (Willadino et al., 1999). This genotypic variability represents a promising approach for identifying AQPs that are functionally relevant for salt tolerance and may serve as potential molecular markers or targets for breeding programs.

This study addresses this gap by analyzing the expression of AQP genes in maize roots exposed to salt stress over a 10-day period, providing new insights into the molecular mechanisms of water transport regulation in maize under saline conditions, and supporting the identification of candidate genes for breeding salt-tolerant cultivars. The transcriptome data was obtained from Chen et al. (2020), who performed a comprehensive analysis of salt stress responses and glycine betaine mitigation in maize seedlings. However, AQP genes were not specifically addressed in their study. Here, we reanalyzed their dataset with a focus on aquaporin expression to explore their potential roles in salt tolerance mechanisms and to identify candidate genes that may contribute to improved stress resilience in maize.

## **MATERIAL AND METHODS:**

### **Experimental conditions of transcriptomes used in gene expression analyses**

In this study, we partially reanalyzed public transcriptome data from Chen et al. (2020). The experimental conditions included two treatments: control (0 mM NaCl) and salt stress (180 mM NaCl). According to the original study, seeds from two maize inbred lines, P138 (salt-sensitive) and 8723 (salt-tolerant), were sown in pots with vermiculite under both conditions and grown in a greenhouse (12-h photoperiod, 600  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  light intensity, 60% humidity) until the three-leaf stage (~10 days). Root samples were then washed, rapidly frozen in liquid nitrogen, and used for RNA extraction, transcriptome generation and sequencing.

### **Genes/cDNAs from aquaporin in *Zea mays***

The 41 aquaporin genes in *Zea mays* were originally characterized by Bari et al. (2018). AQP-related cDNA sequences were retrieved from the RefSeq RNA database (NCBI) and used to study gene expression under salt stress from transcriptomic data [BioProject PRJNA611672 (Chen et al., 2020)].

### **Gene expression analyses in transcriptome data of *Zea mays* genotypes**

Gene expression analysis based on the raw transcriptomic data from Chen et al. (2020) was conducted using three biological replicates per sample and a three-step workflow. First, RNA-seq reads were aligned to the target cDNA sequences using Magic-BLAST (Boratyn et al., 2019). Second, the aligned reads were quantified with HTSeq (Anders et al., 2015). Third, read counts were normalized across samples. In the alignment step, the target cDNAs were used as reference sequences. Gene expression levels were normalized as RPKM (Reads Per Kilobase per Million; Mortazavi et al., 2008). Total expression per subfamily (NIP, PIP, SIP, TIP) was computed by summing RPKM values of each member.

### **Statistical analysis**

Gene expression differences among aquaporin subfamilies (NIP, PIP, SIP, and TIP) were calculated as mean  $\pm$  standard deviation (SD), based on the sum of RPKM values for each subfamily using three biological replicates. Comparisons between control and salt stress conditions for each subfamily were performed using Student's *t*-test at a 5% significance level, using GraphPad Prism version 5.0.

## **RESULTS AND DISCUSSION:**

### **Total gene expression of aquaporin subfamilies in maize roots under salinity**

Transcriptomic analysis of AQP gene expression in maize roots subjected to salinity stress for approximately ten days revealed notable differences between the two inbred lines analyzed: P138, classified as salt-sensitive, and 8723, identified as salt-tolerant. As illustrated in Figure 1, the expression profiles of the major AQP subfamilies showed contrasting responses between the genotypes. In the salt-tolerant line 8723, there was a marked increase in total transcript levels of NIP and PIP genes under salinity conditions. This suggests a possible upregulation of water and solute transport pathways as an adaptive response to maintain cellular homeostasis under osmotic stress.

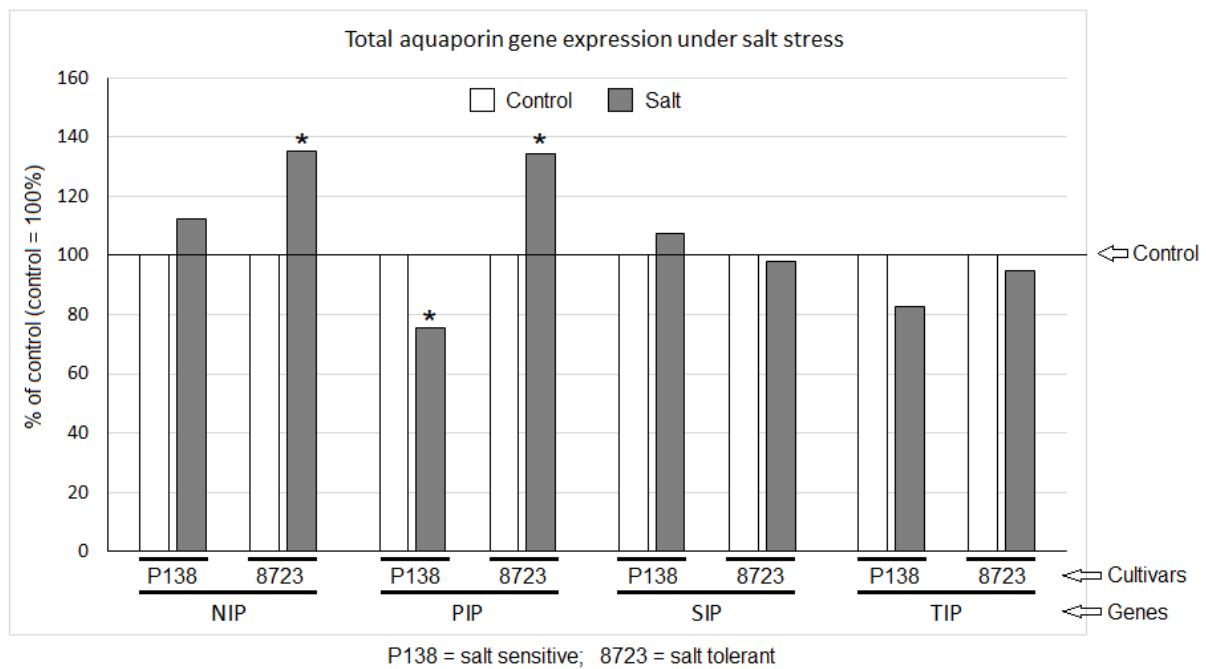
Several studies support the involvement of PIP and NIP aquaporins in salt stress responses. In tropical maize, inoculation with *Pantoea agglomerans* under 0.2 M NaCl led to

up-regulation of PIP genes, particularly PIP2-1, which correlated with enhanced salt tolerance (Gond et al., 2015). Additionally, short-term exposure (2 h) to 100 mM NaCl induced ZmPIP1-1, ZmPIP1-5, and ZmPIP2-4 in maize root outer layers, while a higher salinity (200 mM) repressed multiple PIP and TIP genes (Zhu et al., 2005). In wheat, overexpression of a NIP-type aquaporin (TaNIP) in *Arabidopsis* increased salt tolerance, reducing Na<sup>+</sup> accumulation and enhancing osmoprotective solutes and stress gene expression (Gao et al., 2010).

In contrast, the salt-sensitive line P138 exhibited a more limited or even opposite expression pattern: while NIP transcript levels showed a modest increase, PIP transcripts were slightly downregulated, possibly reflecting a reduced capacity to maintain efficient water transport under stress. Interestingly, the expression levels of SIP and TIP genes showed less variation between the two genotypes, indicating that these subfamilies may be regulated in a more conserved or less stress-responsive manner in maize roots (Figure 1).

Indeed, TIP aquaporins, which localize to the tonoplast and are known to facilitate rapid vacuolar osmotic adjustment, have been reported to respond less dynamically to salinity compared to PIPs, suggesting a more stable role in maintaining cellular osmotic balance under salt stress (Afzal et al., 2016; Kapilan et al., 2018). Meanwhile, although SIPs remain poorly characterized in terms of stress responses, their limited structural diversity and conservative expression patterns across conditions hint at a constitutive or housekeeping function, rather than a stress-inducible one (Kapilan et al., 2018).

Together, these findings suggest that the transcriptional regulation of AQP genes under salt stress is influenced not only by genetic background, but also by the functional specialization and subcellular localization of different AQP subfamilies. Such genotype-dependent regulation may contribute to the differential salt tolerance observed between the two inbred lines.



**Figure 1.** Relative transcript levels of aquaporin genes in maize roots under salt stress (NaCl) from two maize inbred lines, P138 (salt-sensitive) and 8723 (salt-tolerant). Bars represent the total transcript accumulation (percentage) of aquaporin gene families (NIP, PIP, SIP, and TIP) under control (white bars) and salt (gray bars) conditions. Data are shown as percentage relative to control levels for each gene family and cultivar. Asterisks indicate statistically significant differences between control and salt-treated samples at  $p < 0.05$ .

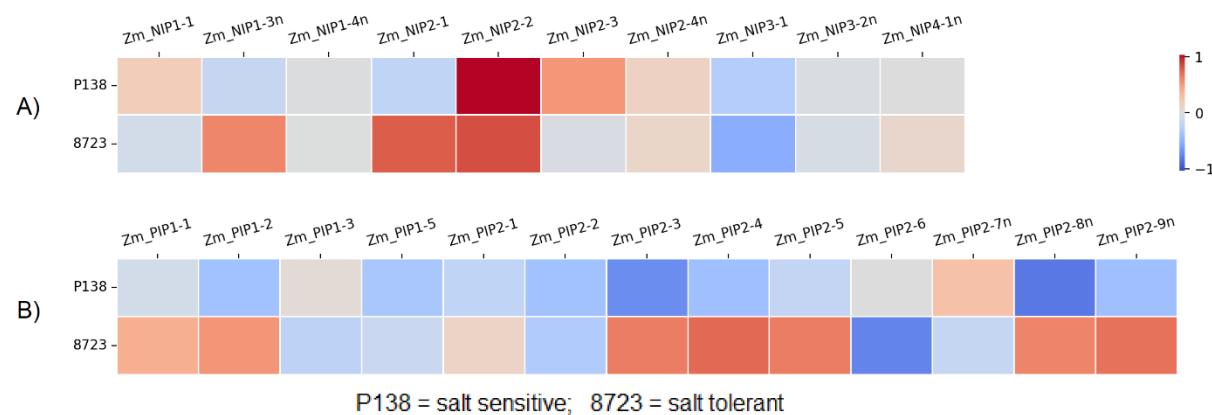
### Main specific aquaporin genes involved in salinity tolerance

Figure 2 highlights a subset of specific aquaporin genes whose expression patterns appear to be closely associated with salinity tolerance mechanisms in maize. In the salt-tolerant cultivar 8723, several genes from the NIP and PIP subfamilies exhibited significant upregulation following ten days of salt stress. These include NIP1-3n and NIP2-1, as well as multiple PIP isoforms, such as PIP1-1, PIP1-2, PIP2-3, PIP2-4, PIP2-5, PIP2-8n, and PIP2-9n. This consistent upregulation suggests that these aquaporins play an active role in facilitating adaptive physiological responses under saline conditions. In contrast, the same genes showed either downregulation or no significant change in expression in the salt-sensitive cultivar P138, indicating a markedly different transcriptional response to salinity.

Our findings in maize, particularly the significant upregulation of NIP and multiple PIP isoforms in the salt-tolerant cultivar 8723, align with and expand upon previous studies in maize and other plant species. In maize, treatment with *Pantoea agglomerans* under salinity (0.2 M NaCl) similarly induced PIP genes, especially PIP2-1, correlating with enhanced salt tolerance (Gond et al., 2015). Similarly, overexpression of ZmPIP2-5 was shown to enhance leaf

hydraulic conductance under drought and mild osmotic stress, further supporting the functional role of these genes in abiotic stress adaptation (Kurowska, 2021). In other cereals, such as wheat, transgenic expression of TaAQP8 (a PIP1 aquaporin) enhanced salt tolerance by promoting root growth and maintaining ionic homeostasis. Beyond cereals, the aquaporin MaPIP1-1 from banana was strongly induced by salt stress, and its overexpression in transgenic *Arabidopsis* improved tolerance by reducing membrane damage and enhancing osmotic adjustment (Xu et al., 2014).

This differential gene expression between the two genotypes reinforces the hypothesis that specific members of the NIP and PIP subfamilies are key contributors to the regulation of water and solute transport under stress. Their upregulation in cultivar 8723 likely promotes improved osmotic adjustment, enhanced ion balance, and sustained root hydraulic conductivity, which are critical for maintaining cellular function and water status during salt stress. Conversely, the lack of induction or repression of these genes in P138 may limit the plant's ability to cope with osmotic and ionic challenges, leading to reduced water uptake and increased sensitivity to saline environments. Overall, these findings support the view that aquaporin-mediated transport processes are central to the mechanisms that differentiate salt tolerance levels among maize genotypes.



**Figure 2.** Heatmap of Log2 Fold Change of specific gene members of NIPs (A) and PIPs (B) in maize roots under salt stress (NaCl) from two maize inbred lines, P138 (salt-sensitive) and 8723 (salt-tolerant). The heatmap shows differential expression patterns of key aquaporin gene members under salt stress compared to control conditions.

## CONCLUSION:

The transcriptomic analysis of maize roots exposed to salinity stress revealed distinct differences in the expression patterns of aquaporin genes between the salt-tolerant inbred line 8723 and the salt-sensitive line P138. In response to ten days of salt exposure, the tolerant

cultivar 8723 demonstrated sustained or enhanced expression of several key genes, particularly NIP1-3n, NIP2-1, PIP1-1, PIP1-2, PIP2-3, PIP2-4, PIP2-5, PIP2-8n, and PIP2-9n. In contrast, the sensitive cultivar P138 exhibited either downregulation or no significant change in the expression of these same genes. This contrasting expression pattern strongly supports the idea that specific members of the NIP and PIP subfamilies are involved in the adaptive response to salinity stress.

These aquaporins likely contribute to improved water uptake, osmotic adjustment, and cellular homeostasis, which are critical for plant survival under salt-affected conditions. Their selective upregulation in the tolerant genotype indicates a potential mechanistic basis for enhanced stress resilience, making these genes promising molecular markers or targets for genetic improvement or breeding programs aimed at improving salinity tolerance in maize. Overall, the findings of this study provide important insights into the molecular responses of maize to salinity and highlight the value of aquaporin gene expression profiling in the development of salt-tolerant maize cultivars, particularly in regions affected by increasing soil salinization.

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