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Research article

# Genome-wide analysis and expression profiling of the *Solanum tuberosum* aquaporins

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ABSTRACT

Aquaporins belongs to the major intrinsic proteins involved in the transcellular membrane transport of water and other small solutes. A comprehensive genome-wide search for the homologues of *Solanum tuberosum* major intrinsic protein (MIP) revealed 41 full-length potato aquaporin genes. All potato aquaporins are grouped into five subfamilies; plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs) and x-intrinsic proteins (XIPs). Functional predictions based on the aromatic/arginine (ar/R) selectivity filters and Froger's positions showed a remarkable difference in substrate transport specificity among subfamilies. The expression pattern of potato aquaporins, examined by qPCR analysis, showed distinct expression profiles in various organs and tuber developmental stages. Furthermore, qPCR analysis of potato plantlets, subjected to various abiotic stresses revealed the marked effect of stresses on expression levels of aquaporins. Taken together, the expression profiles of aquaporins imply that aquaporins play important roles in plant growth and development, in addition to maintaining water homeostasis in response to environmental stresses.



## 1. Introduction

In plants, water transport comprises a combination of apoplastic (cell wall and extracellular space) and symplastic (plasmodesmata and transmembrane) pathways. Transcellular movement of water is controlled by aquaporins, a special class of transmembrane channel proteins that belong to the major intrinsic proteins (MIPs) superfamily [1,2]. Aquaporins are present in all living organisms and their distribution greatly varies with cellular complexity. Unlike animals or microbes, plants are known to express a larger number of aquaporins. For example, 35 homologues in *Arabidopsis thaliana* [2], 36 homologues in *Zea mays* [1], 33 homologues in *Oryza sativa* [3], 65 homologues in *Populus trichocarpa* [4], and 68 homologues in cotton [5] were identified through genome sequencing and expressed sequence tag data. Based on the sequence homology and subcellular localization, MIPs gene family in plants is classified into five subfamilies; plasma membrane intrinsic protein (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs) and x-intrinsic proteins (XIPs). However, the, XIP subfamily is not encoded by the genomes of monocots and, certain plant species in dicots, such as *Arabidopsis* [4,6].

Aquaporins occur as tetrameric complexes in which each subunit behaves as a functional water channel [7]. Each monomer consists of six transmembrane (TM) spanning α-helices (TM1–TM6) that are connected by five interhelical loops (A–E), with both the amino and the carboxyl termini located on the cytoplasmic side of the membrane. The pore region of the channel is characterized by two highly conserved NPA (Asn-Pro-Ala) motifs, located in loop B and E. Overlapping of the two NPA motifs in the middle of the lipid bilayer forms one of the two channel constrictions sites involved in proton exclusion and channel transport selectivity [8]. The second constriction is referred to as the aromatic/arginine (ar/R) constriction or the selectivity filter and is formed at the extracellular side of the pore by four residues from TM helix 2 (H2), TM helix 5 (H5), and loop E (LE1 and LE2), respectively. Variability at this site is thought to form the basis of the broad spectrum of solute transport in plant aquaporins [4,9–11].

Aquaporins are known to play a major role in maintaining water balance in the plant system [7]. Recent advances suggest that plant aquaporins plays a central role in numerous physiological processes, such as plant–soil water relations, seed germination,

*Abbreviations:* AQPs, aquaporins; GLPs, quaglyceroporins; MIPs, major intrinsic proteins; NIPs, NOD26-like intrinsic proteins; NJ, neighbour-joining; PGSC, potato genome sequencing consortium; PIPs, plasma membrane intrinsic proteins; SIPs, small basic intrinsic proteins; TIPs, tonoplast intrinsic proteins; XIPs, x-intrinsic proteins.

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transpiration, closure of leaf guard cells, tree embolism recovery, fruit ripening, petal movement and anther dehiscence, cell elongation, maintenance of cell turgor and various abiotic stresses [7,12,13]. Aquaporins also play a key role in transportation of mineral nutrients, such as boron and silicon or metalloids, such as arsenate [14,15]. Furthermore, aquaporins promote carbon and nitrogen fixation through their involvement in the passage of a wide range of small neutral molecules, such as carbon dioxide, ammonia and urea [16–18].

Given the potential value of aquaporins in improving stress tolerance, we screened the annotation of *Solanum tuberosum* group phureja DM1-3 version 2.1.10 (http://potato.plantbiology.msu.edu/cgi-bin/gbrowse/potato/) and identified 41 aquaporin genes representing all five aquaporin subfamilies. To our knowledge, this is the first genome-wide study of the potato aquaporin gene superfamily and using systematic nomenclature grouped the aquaporin genes into subfamilies based on sequence similarity and phylogenetic relationship with their *Arabidopsis*, rice and cotton counterparts. Furthermore, the structural properties and functional components of potato aquaporins were investigated using computational tools. Finally, detailed qPCR expression analysis was carried out for the potato MIP genes under abiotic stresses i.e. drought, salt, heat and abscisic acid (ABA) and in various tissues of the potato.

## 2. Results

### 2.1. Potato aquaporins identification and classification

Based on sequence homology analysis, several aquaporin-like genes were identified from the *S. tuberosum* genome. Genes encoding partial aquaporin-like sequences, which are truncated and lacking any of the NPA motifs, were considered non-functional pseudogenes and were excluded from the sequence analysis. Similarly, several truncated splice variants were also excluded from the sequence analysis (Table S1 and S2, Supplementary data). Consequently, 41 full-length protein-coding aquaporin genes were identified in the potato genome. The predicted amino acid sequence of the potato aquaporins was used to perform a phylogenetic analysis. Overall, potato aquaporins followed the phylogenetic pattern in a similar manner to their *Arabidopsis*, cotton and rice counterparts and clustered into five distinct subfamilies; PIP (15), TIP (11), NIP (10), SIP (3) and XIPs (8) (Figs. 1 and 2). The potato PIP subfamily were further divided into two phylogenetic subgroups; PIP1 (5) and PIP2 (9). TIPs were divided into five subgroups (TIP1 to TIP5). The NIP subfamily was divided into four subgroups (NIP1, NIP3, NIP4 and NIP5) with no NIP2 homologue members identified. The potato SIP subfamily included only one subgroup (SIP1) whereas the XIP subfamily included four subgroups (XIP1 to

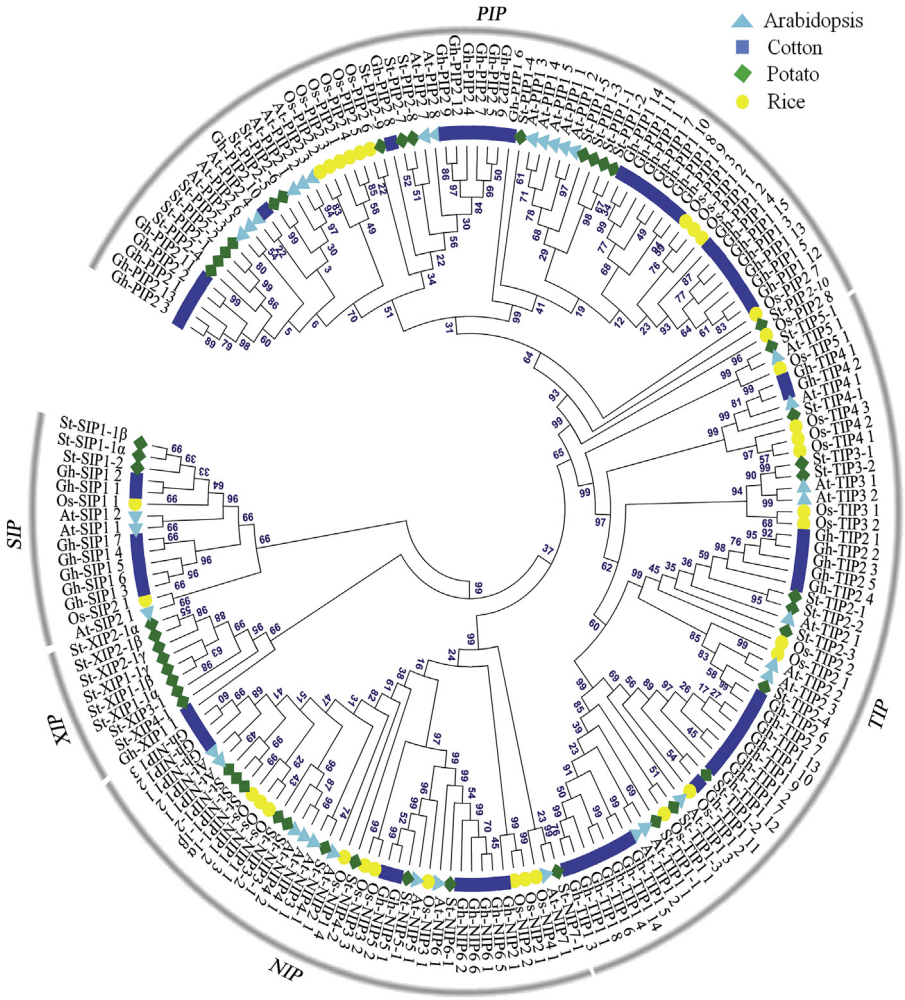


**Fig. 1.** Phylogenetic analysis of the predicted amino acid sequences of potato aquaporins with members of other plant species. Predicted amino acid sequences were aligned using ClustalW2 sequence alignment program and the phylogenetic tree was constructed using Bootstrap NJ tree (1000 replicates) method and MEGA5 software. The name of each subfamily is indicated next to the corresponding group.