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Genome-Wide Identification, Characterization, and Expression Profiling of The Aquaporin Gene Family in *Panax ginseng*

Ick-Hyun Jo* , **Chi-Eun Hong, Woojong Jang, Jung-Woo Lee, Jang Uk Kim, and Kyong-Hwan Bang**

Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, Eumseong 27709, Korea

ABSTRACT

Water movement across cellular membranes is mostly regulated by aquaporins (AQPs). The AQPs have been found to play an important role in plant growth and development, and also in the response of plants to abiotic stress. Despite the vital role of AQPs, the regulation of its function and activity remains unknown in *Panax ginseng*. In this study, we identified 21 aquaporin unigenes from *P. ginseng* Iso-Seq data that were separated by phylogenetic analysis into three sub-families (PIP, TIP and SIP). Next we selected a total of 10 unigenes based on criteria including the elimination of overlapping TAIR ID, the longest ORF, and e-value. To identify relative expression patterns to salt stress, 150 mM NaCl was treated with 3-week-old ginseng adventitious roots and the treated tissues were collected at the indicated time point for RNA extraction. Expression patterns varied however transcript level of 5 genes (KG_ISO002645, KG_ISO_037543, KG_ISO_040118, KG_ISO_12789, KG_ISO_132560) increased at early time points (6, 9, 12 h) compared to control after salt stress. To confirm the sub-cellular localization of PgAQPs predicted using the online tool WoLF PSORT tool, the AQP:GFP fusion constructs were constructed. Subcellular localization analysis in *Nicotiana benthamiana* epidermal cells revealed the diverse and broad array of sub-cellular localizations of *Panax ginseng* aquaporins (PgAQ4, SIP1; PgAQ6, TIP1; PgAQ14, PIP2). These results can also further expand our understanding of the AQPs in *P. ginseng* and may contribute to genetic engineering for ginseng cultivar stress-resistance improvement.

RESULTS

Table 1. The unigenes representing homolgues most similar to Arabidopsis genome in this study.

PG ID	Unigene (bp)	CDS (bp)	Arabidopsis ID	TAIR description	E-value
KG_ISO_002474	2,124	843	AT4G35100	PIP3, PIP3A, PIP2;7, SIMIP | plasma membrane intrinsic protein 3	4.00E-115
KG_ISO_002645	1,136	708	AT3G54820	PIP2D, PIP2;5 | plasma membrane intrinsic protein 2;5	3.00E-91
KG_ISO_008379	1,742	960	AT4G10380	NIP5;1, NLM6, NLM8 | NOD26-like intrinsic protein 5;1	2.00E-137
KG_ISO_014387	1,195	864	AT4G00430	TMP-C, PIP1;4, PIP1E | plasma membrane intrinsic protein 1;4	0
KG_ISO_025157	1,717	369	AT5G37630	EMB2656 | ARM repeat superfamily protein	4.00E-31
KG_ISO_028867	991	849	AT4G01470	GAMMA-TIP3, TIP1;3, ATTIP1.3 | tonoplast intrinsic protein 1;3	4.00E-108
KG_ISO_037543	874	456	AT4G23400	PIP1D, PIP1;5 | plasma membrane intrinsic protein 1;5	1.00E-97
KG_ISO_040118	1,025	711	AT5G18290	SIP1;2, SIP1B | Aquaporin-like superfamily protein	2.00E-65
KG_ISO_041736	1,480	303	AT1G01620	PIP1C, TMP-B, PIP1;3 | plasma membrane intrinsic protein 1C	2.00E-11
KG_ISO_044257	983	762	AT2G36830	GAMMA-TIP, TIP1;1, GAMMA-TIP1 | gamma tonoplast intrinsic protein	1.00E-115
KG_ISO_049746	2,388	678	AT1G80760	NIP6;1, NIP6, NLM7 | NOD26-like intrinsic protein 6;1	1.00E-63
KG_ISO_052030	3,043	534	AT5G67360	ARA12 | Subtilase family protein	8.00E-27
KG_ISO_055155	1,308	846	AT2G37170	PIP2B, PIP2;2 | plasma membrane intrinsic protein 2	2.00E-167
KG_ISO_063661	1,050	546	AT3G06100	NLM6, NLM8, NIP7;1 | NOD26-like intrinsic protein 7;1	1.00E-45
KG_ISO_068417	3,238	999	AT5G47380	Protein of unknown function, DUF547	2.00E-06
KG_ISO_085376	4,146	642	AT3G05970	LACS6, ATLACS6 | long-chain acyl-CoA synthetase 6	4.00E-74
KG_ISO_092029	3,757	423	AT2G34380	Putative adipose-regulatory protein (Seipin)	1.00E-74
KG_ISO_125127	8,940	345	AT3G05680	EMB2016 | embryo defective 2016	7.00E-14
KG_ISO_126789	1,240	837	AT5G60660	PIP2F, PIP2;4 | plasma membrane intrinsic protein 2;4	2.00E-164
KG_ISO_132560	1,051	690	AT5G47450	ATTIP2;3, TIP2;3, DELTA-TIP3 | tonoplast intrinsic protein 2;3	9.00E-128
KG_ISO_134602	989	510	AT3G04090	SIP1;1, SIP1A | small and basic intrinsic protein 1A	4.00E-46

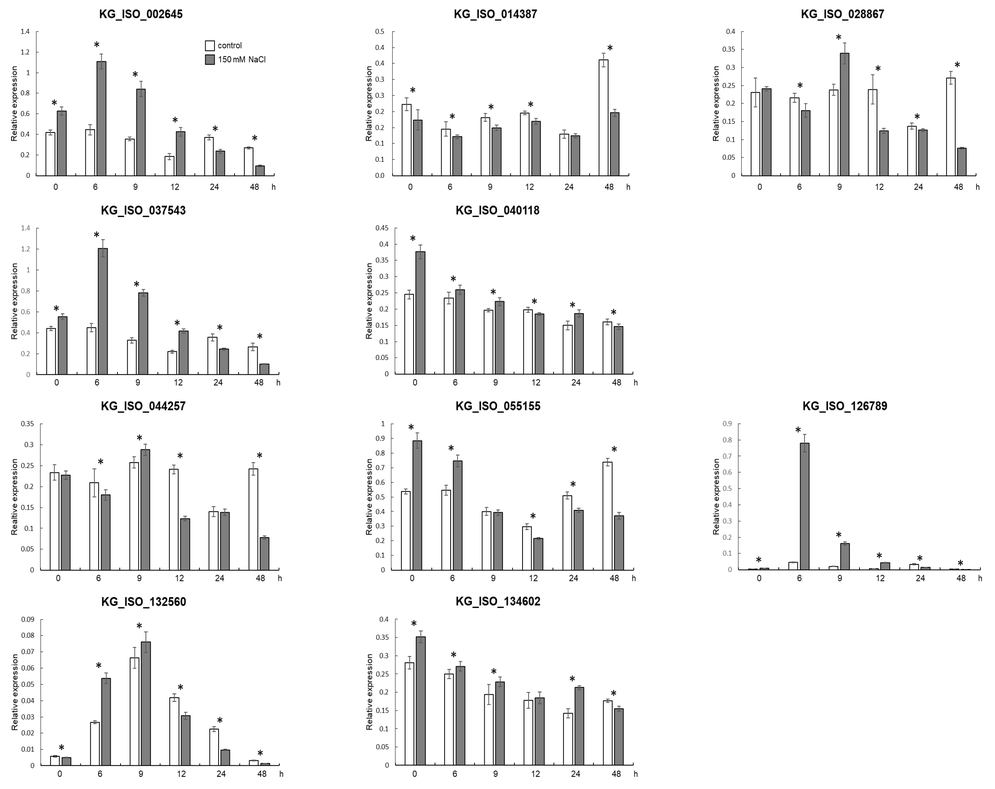

Figure 1. Expression of the putative aquaporin genes in ginseng adventitious roots. Three-week-old adventitious roots were treated with 150 mM NaCl. Sterile distilled water was used as a control. Total RNA was extracted from ginseng adventitious roots at the indicated time points. Asterisk indicates statistical difference(* P<0.05) compared to control.

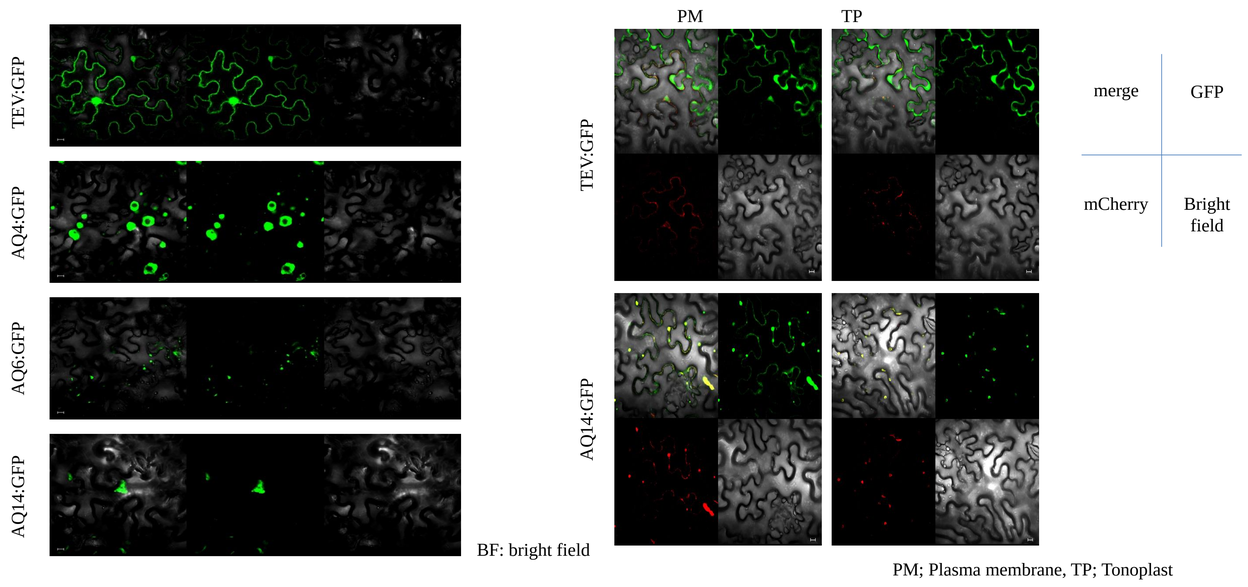

Figure 2. Subcellular localization of PgAQPs. The free GFP (positive control), as well as the AQP:GFP fusion proteins of AQ4; PgSIP1, AQ6; PgTIP1, and AQ14; PgPIP2, were transiently expressed in tobacco leaves via *Agrobacterium tumefaciens* strain GV3101. Subcellular localization was then observed by confocal laser scanning microscopy after 48 h from the infiltration. Scale bar = 20 μm.

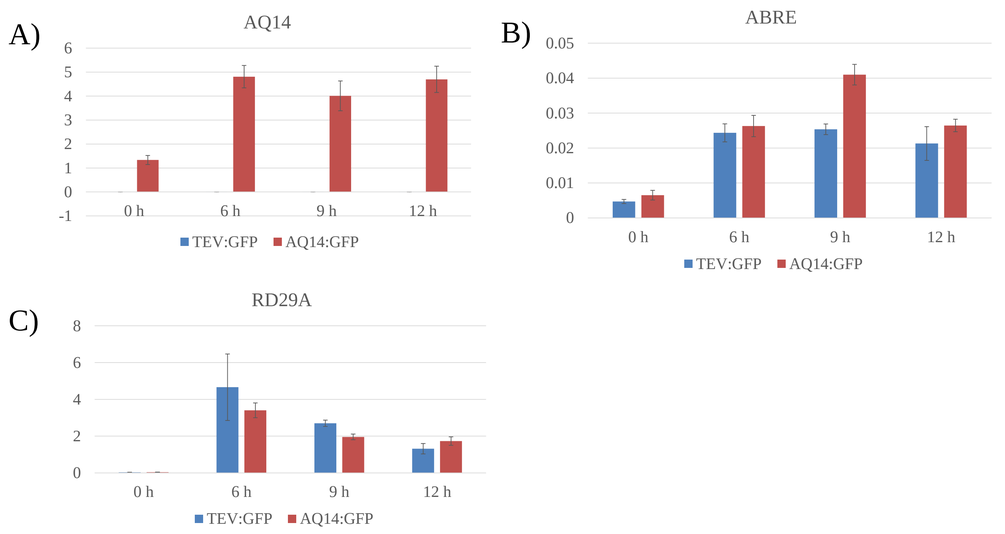

Figure 3. Expression patterns of AQ14, ABRE and RD29A in *Arabidopsis thaliana* plants. (A) Overexpression of AQ14(PgPIP2) in *Arabidopsis*. Expression patterns of ABRE (B) and RD29A (C) in of transgenic *Arabidopsis thaliana* plants. Time course analysis of AQ14(PgPIP2), ABRE and RD29A expression in response to drought stress. Each column represents means ± SD from three replicates.

CONCLUSION

This A total of 10 AQP genes in three sub-families were identified and characterized based on their sequences, phylogenetic relationships, and expression profiles upon abiotic stress. qRT-PCR analysis showed that salinity stress could alter the expression levels of PgAQPs. Moreover, transient expression analysis indicated that AQPs play roles in the regulation of plant water status. Under stress conditions, further molecular study of PgAQPs should reveal more functional mechanisms for these genes. These results can also further expand our understanding of the AQPs in *P. ginseng* and may contribute to genetic engineering for ginseng cultivar stress-resistance improvement.

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Corresponding author: Ick-Hyun Jo / Tel. 043-871-5613 / E-mail: intron@korea.kr