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Research Article

In Silico Genome wide identification and analysis of Aquaporins in Barley (*Hordeum vulgare* L.)

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Abstract

Aquaporins (AQP) are essential membrane proteins that play a significant role in the transport of water and other small molecules across membranes. Water transport rates can be quickly reduced by environmental challenges such as salt, drought, and low root temperature and Barley is the fourth most significant cereal crop in the world, behind maize, wheat, and rice. Since the dawn of humanity, it has been one of the earliest domesticated food crops. However, currently little is known about the AQP genes in Barley (*Hordeum vulgare*). In this study, a comprehensive genome-wide analysis identified 44 AQP genes in Barley, according to their chromosomal location, gene ID, length, coordinates, splice variants, and subcellular location. The highest chromosome number of this domain is 7 and the least chromosome number is 1. The classification was further validated by gene structure, sequence identity, conserved motif, and phylogeny analysis. It presents the genome wide analysis of the AQP gene in Barley and provides valuable information for further functional analysis to infer the role of AQP in the adaptation of Barley in diverse environmental conditions.

Keywords: Genome-wide, Aquaporins, AQPs, Barley, Hordeum vulgare

1. Introduction

Hordeum vulgare L., also known as barley. In India, barley is the second most significant winter cereal after wheat in terms of both acreage and production. Barley is effectively cultivated in adverse agro-environments such as drought, salt, and alkalinity in a variety of topographical circumstances such as plains and hilly places under rainfed and irrigated settings due to its resilient nature. Because of its high input requirements, barley is commonly referred to be a poor man's crop. Approximately 90% of the barley grown in India is consumed by humans. It has roughly the same nutritional content as kernel corn as a feed. It's particularly useful as a feed since it produces ideal chunks of firm fat. After grinding or steam rolling, the entire kernel is used in feed. Malt sprouts and brewers' grain, which are byproducts of brewing, are also useful livestock feeds. The barley crop is also used to make malt. Malted barley is primarily utilised in the production of beer, distilled alcohol, malt syrup, malted milk, and breakfast items.

(AQPs) are membrane channel proteins found in plants and other creatures that selectively and reversibly allow water transport across plasmalemma and organelle membranes. Water is constantly absorbed by roots, flowing axially through xylem vessels, and travels radially to leaves via apoplastic, symplastic, and transcellular pathways before evaporating through stomata in growing plants. Water passes cellular membranes in the transcellular channel either by simple diffusion or, more commonly, by AQP-formed holes. Under many circumstances, such as during cell elongation, root water absorption, leaf movement, stomatal opening and closure, flowering,

and fertilisation, the rapid movement of water through the biomembrane is required for the maintenance of cellular water homeostasis as well as the accomplishment of various metabolic activities. As a result, AQPs play a critical role in plant growth, development, and survival.

Hordeum vulgare L. is a cereal plant species in the *Hordeum* genus and *Poaceae* family (Muntean et al., 2014). Barley has gradually acquired characteristics that have aided agricultural production during the domestication process. Initially, natural selection was carried out by the environment, and later, artificial selection was carried out by man (Bothmer et al., 2003). Barley comes in a variety of varieties, each having a varied amount of grains per spike, ranging from summer to winter barley. Due to the poor quality of the grain, winter barley is mainly used in animal feed.Barley is a cereal grain used for a variety of purposes, including feed, beer production, and food[1]. Barley has medicinal capabilities, which are boosted by the treatment of various conditions, in addition to the benefits already mentioned. Barley, along with wheat and millet, was one of the most important crops of the period. Barley's value has waned in recent decades, and its planted area has shrunk by three to four times.

H. spontaneum, the wild parental type from which cultivated barley evolved, is still prevalent in its natural habitats in Israel, Jordan, southern Turkey, Kyrgyzstan, and the south-west of Iran (Harlan and Zohary, 1966; Russu, 2015). Similar ecosystems that H. spontaneum has lately colonised include the Aegean, south-east Iran, Central Asia, including Afghanistan, and the Himalayan areas (Russu, 2014, 2015). H. spontaneum is also found in Greece, Egypt, southeast Asia, and southern Tajikistan, according to Bothmer et al. (1995).Secondary barley domestication centres include the Himalayas, Ethiopia, and Morocco (Aberg (1938), cited by Bothmer and Komatsuda, 2011; Bekele, 1983; Russu, 2015)[2]. Barley is a food with a low glycemic index (GI) of less than 55. Gluten-free and diabetic patients can both eat barley-based dishes because of its value. For those with type II diabetes or prediabetes, barley is an excellent source of nutrition. This grain is high in soluble dietary fibre, particularly beta glucans, and is a good source of vital vitamins and minerals (www.Barleyfoods.org). According to studies, barley grains contain 60-65 percent starch, 10-17 percent protein, 4-9 percent beta-glucans, 2-3 percent lipids, 1.5-2.5 percent mineral compounds, and 3-20 percent soluble fibres (Czuchajowska et al., 1998; Quinde et al., 2004) [3]. The chemical composition of barley grains differs from green barley's chemical composition. [4]

Green barley's molecular makeup has a unique therapeutic impact on the plant, reducing the ageing process of the cells. When green barley reaches a height of 20-30 cm, it has the best nutritious resources for human cells. B vitamins (B1, B2, B6, and B12), vitamin E, and vitamin C are abundant in this phenophase, as well as iron, calcium, manganese, magnesium, molybdenum, germanium, zinc, copper, lithium, bioflavonoids, polysaccharides, and polypeptides[5].

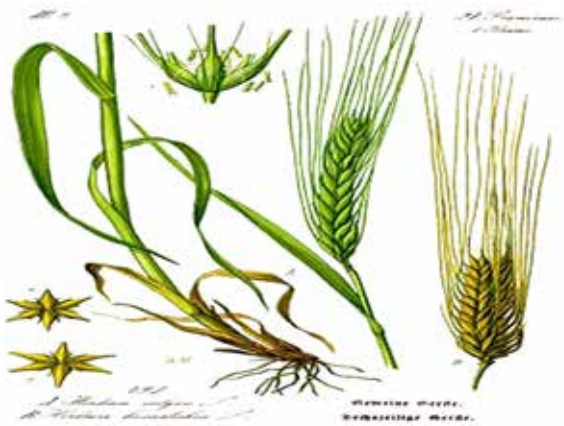


Figure1. Barley(https://en.wikipedia.org/wiki/Barley)

The high concentration of these components in barley makes it effective in maintaining body function. Barley is utilised in medicine and therapy as a powder or fresh juice (green barley), or as barley water. Barley, green. Barley is utilised in medicine and therapy as a powder or fresh juice (green barley) or as barley water. Green barley is the juvenile leaf of the barley plant, commonly known as 'barley grass.' Following a research of 150 different plants, Dr. Yoshihide Hagiwara, a doctor in medicine, identified the significant impacts of green leaves on human health. Green barley has proven to be the most beneficial to the human body, as well as having a strong therapeutic impact (Hagiwara, 1985, 1995).

When green barley reaches a height of 20-30 cm, before the plant stem has matured and grains have formed, the best nutritious content for human cells is reached. For the best results, Dr. Hagiwara suggests using barley essence (1985). The green barley essence is made by dehydrating fresh green barley juice at low temperatures, which

keeps the enzymes alive. Comparisons between green barley essence and numerous popular foods (Hagiwara, 1985) in terms of primary components of the examined elements , mineral content, and vitamin content reveal the benefits of green barley. Barley has a key role in the control of a wide range of nutritional, cardiovascular, and cancer disorders, as well as a biorenewable alternative energy generator, thanks to its vast number of bioactive chemical components (Russu, 2015). Because it decreases cholesterol and blood glucose levels, -glucan is very useful for human intake (Russu, 2015).

According to Behall (2004), referenced by Russu (2015), barley consumed in the form of various foods high in soluble fibre reduces the occurrence of cardiovascular illnesses. Soluble fibres ferment in the colon, resulting in small, 91-chain fatty acids that can be absorbed and prevent hepatic cholesterol formation. There have been numerous scientific studies on the health benefits of green barley, including cancer prevention (Woo et al., 2017; Kawka et al., 2019), hyperlipidemia and cardiovascular disease (Behall et al., 2004; Talati et al., 2009; Ho et al., 2016; Elmhdwi et al., 2018), and other chronic diseases (Shibamoto, 1997; Benedet et al (Zeng et al., 2018).

Anemia, cystitis, rheumatism, diarrhoea, cough, haemorrhoids, flu, sterility, depressed conditions, dermatological problems, and other conditions are all treated by green barley. Barley is also valued for its remineralizing, anti-inflammatory, hypotensive, and sexual appetite-inducing characteristics, as well as its ability to aid recovery after a convalescent period (www.wikipedia.org). Egyptians have utilised barley water for health and strength since ancient times. The traditional beverage has long been used to treat gastroenteritis. There are also reports of people drinking barley water to treat nausea and mouth sores induced by chemotherapy side effects (Newman and Newman, 2008). Candies produced with barley water, which have the reputation of being soothing for throat and intestinal ailments, were also a typical Victorian treat (Newman and Newman, 2008). Created with barley grains, cold water, sugar or honey, salt, and lemon, barley water can be made at home.

The important effects of the green leaves on human health, was discovered by Dr.Yoshihide Hagiwara, doctor in medicine, after a study of 150 different plants. Of all, green barley proved to be the most valuable for human body and also has an excellent therapeutic effect (Hagiwara, 1985, 1995).

One of the main reasons for the rise in barley farming during the Mesopotamian period could have been barley's stronger resistance to salt stress than wheat. In plants, the main function of AQPs is to regulate transmembrane water transport in cases where the flow needs to be altered or is severely low [7]. AQPs can be found in practically every organ of plants, including roots, leaves, stems, flowers, fruits, and seeds [8,9]. Changes in a plant's hydraulic conductivity can be influenced in part by AQP activity, particularly PIPs [10,11]. According to several studies, AQPs can influence water flow across cell membranes by changing AQP abundance or changing the water flow rate [12].The interaction of AQPs with other physiological and metabolic processes in the plant can affect their activity [13]. Plant AQPs differ in substrate specificity, location, transcriptional and posttranslational regulation, and substrate specificity [14, 15]. Plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26 like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and X intrinsic proteins/uncharacterized-intrinsic proteins (XIPs) are the five subfamilies of AQPs in higher plants [16] based on membrane localization and amino acid sequence. Plants have a higher number of AQP genes than animals, ranging between 30 and 70 [20,15,21]. In poplar, for example, 55 AQP genes have been discovered [19,22]. Under abiotic stress, reactive oxygen species (ROS) govern plant signalling responses; yet, they can cause severe oxidative damage at the tissue and cellular levels. High light intensity has been shown to stimulate the generation of ROS in parenchyma cells, resulting in reversible oxidative gating of AQPs [28,29]. There have been two separate ROS regulation mechanisms proposed. The activity of AQP can be influenced by oxidising cysteine residues in the pores, which causes conformational changes in the protein and, as a result, the channel is closed by dephosphorylation [7].Through the oxidation of lipids, *OH can target the triple bond structures between the carbons of the plasma membrane, and the resultant lipid radicals will seal the AQP hole [29]. Apoplast can also create H2O2 in response to ABA and other stressful environmental factors including dryness and salinity [30]. It's also been proposed that during stress, ROS can be created in excess, causing signal tr AQP abundance is regulated by a variety of

developmental and environmental factors, including biotic and abiotic stressors, according to numerous studies. Water transport rates can be quickly reduced by environmental challenges such as salt, drought, and low root temperature [31,32,33,34,35]. It is obvious that maintaining water balance under stress conditions can be a challenging and critical problem for plants.As a result, plants must have a variety of adaptive responses to deal with environmental challenges and their implications for water balance.Under various environmental stress circumstances, AQPs play an important role in maintaining water homeostasis and balance [7].To govern water fluxes inside the cells and in and out of the cells, the abundance and activity of AQPs in the plasma membrane is regulated.

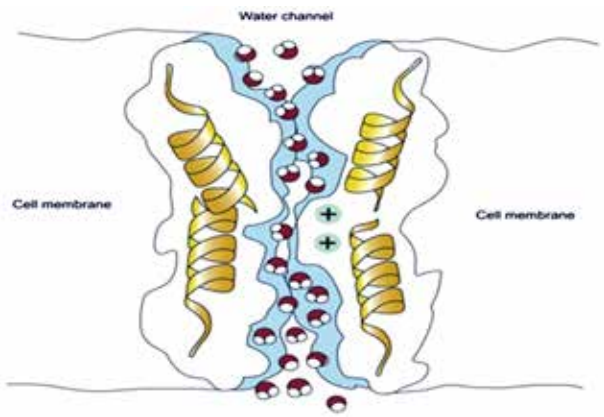


Figure2. Aquaporins (https://qph.cf2.quoracdn.net/main-qimg-4a2ea1f6f9fc9d6f87e4b91323dad0fc-lq)

Drought and salinity, for example, can alter transmembrane water flow[35]. Changes in AQP expression can be utilised to control water transport in response to various abiotic conditions. However, depending on the plant growth conditions, stages of development, and tissue type, as well as the duration and degree of stress and the kind of AQP,its efficiency can vary.Specific AQP isoforms may be expressed in specific tissues during abiotic stress, while other isoforms are expressed throughout the plant. Manipulation of AQP gene expression has aided in the understanding of plant water interactions under stress. Molecular studies of the AQP family's regulation have frequently found complex transcriptional and posttranslational responses, with occasionally opposing patterns between isoforms. The amount of AQP transcripts and the encoded proteins, on the other hand, are not always associated [13,22].Because of protein turnover and posttranslational modifications, not all AQP transcripts are translated into proteins (PTM). Following initial protein synthesis, polypeptides undergo structural and functional alterations in a process known as PTM. The equilibrium between protein production and breakdown is known as turnover.Because distinct AQP genes may be activated, decreased, or remain unaltered under abiotic challenges, the exact role of AQPs in maintaining plant water status under varied stress conditions cannot be fully defined.Although the function of AQPs in rice was not closely associated with root water fluxes, they were increasingly critical under drought stress circumstances. AQP membrane trafficking can also be affected by stress. Salt stress was reported to cause PIP2;1 internalisation from the plasma membrane to the vacuolar lumen in Arabidopsis, and this process was inhibited by kinase inhibitors and clathrin-mediated endocytosis inhibitors. The SpAQP1 gene from the halophytic plant Sesuvium portulacastrum conferred stress resistance to tobacco, demonstrating the role of AQPs in salt stress tolerance. Transduction to activate the AQP and block the AQP channel [6,7].

2. Materials and methods

2.1 Data collection

Amino acid sequences of reference genes and TFs will be obtained from different biological databases including NCBI, TAIR, Gramene RAP-DB, etc.

2.2 Identification and chromosome localization of genes and TFs in barley genome

BLASTP algorithm will be carried out against latest barley genome assemblies available on IWGSC-URGI (https://urgi.versailles.inra.fr/) and Ensembl Plants (http://plants.ensembl.org/index.html) databases.

2.3 Functional domains, physicochemical properties and subcellular localization analysis

Conserved domain database (CDD) of NCBI (www.ncbi.nlm.nih.gov) will be utilized to predict the functional domains of osmotic stress responsive genes and TFs. Physicochemical properties including molecular weight (MW), isoelectric point (pI), charge and average residue weight, will be identified by using ProtParam server of Expasy tools (https://web.expasy.org/protparam/)while subcellular localization will be predicted by using PLANT-mPLoc(http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

2.4 Prediction of exon and Intron boundaries

The Intron/Exon boundaries for individual genes and will be determined by aligning the CDS sequences to their corresponding genomic DNA sequences that will be utilized as input for graphical display at the Gene Structure Display Server (GSDS v2.0) of Peking University, China (http://gsds.cbi.pku.edu.cn/) and motif analysis by using MEME tool.

2.5 Homology modeling Protein

Predicted 3D structure deduced by using automated Swiss -Model server were further visualized in CPK by UCSF CHIMERA and then by using PSVS tool.

2.6 Phylogeny analysis

Phylogeny analysis will be performed by multiple sequence alignment (MSA) using ClustalX ver. 2.1. MEGA 6.0 will utilize to obtain the phylogenetic tree using the neighbor-joining method.

3. Results and Discussion

3.1 To genome -wide identify the aquaporin gene in barley

I performed the BLASTP searches against the IWGSC RefSeq v1.0 data available at EnsemblPlants 42.0 (see Materials and Methods).In the present study, we identified 44 different barley loci encoding aquaporins proteins in barley using genome-wide approach. Identified HvAP were filtered by removing redundant sequences and different transcripts of the same gene. To further verify the reliability of these HvAP genes, the amino acid sequences of all 44 proteins were searched for the presence of the conserved aquaporins domain and other associated domains using by InterPro and PROSITE databases. Based on the domain confirmation analysis, the 44 HvAP genes having aquaporin gene id in Table1. Functional domains exist in aquaporins proteins are not only essential for proper functioning but also facilitate their localizations to the cell membrane and vacuole.

The identified 44 HvAP genes were named HvAP1-1 to HvAP44-7 according to their chromosomal positions. The gene ID, length, chromosome location, coordinates, splice variants, and subcellular location of HvAP are listed in Table 1 . Sequence analysis revealed that the coding sequence (CDS) length of deduced Aquaporin genes ranged from 252 (HvAP41-7) to 4155bp (HvAP30-6), and corresponding protein length ranged from 48 (HvAP8-2) to 623aa (HvAP20-4) (Table 1). The calculated molecular weight (Mw) and isoelectric points (pI) ranged from 5291.09(HvAP8-2) to 42276.35g/mol (HvAP35-6) and from 4.9 (HvAP21-4) to 12.12 (HvAP35-6), respectively. Out of 44 HvAP proteins, 28 proteins had stable nature while remaining 16 proteins were found unstable at the level of biological sequences (Table 2). Instability index was calculated ranged from 14.8 (HvAP22-4) to 70.92 (HvAP35-6). The higher aliphatic index of the HvAP proteins ranged from 72.31 (HvAP19-4) to 132.18 (HvAP1-1) suggested higher stability at a wider range of temperatures. While predicted GRAVY score of HvAP proteins ranged from -0.879(HvAP35-6) to 1.058 (HvAP16-3) suggested that nearly all HvAP are hydrophilic in nature (Table 2). Subcellular localization prediction showed that most of the HvAP proteins might be located in the vacuole, cell membrane, cell wall, chloroplast (Table1). The diversity in subcellular localization implies various biological functions within the members of barley aquaporin family.

3.2 Chromosomal distribution and homologs relationship of HvAP

Based on IWGSC -URGI and EnsemblPlants barley genome databases, the physical positions of the HvAP genes to corresponding chromosomes. The highest chromosome number of this domain is 7 that is present in HvAP36-7, HvAP37-7, HvAP38-7, HvAP39-7, HvAP40-7, HvAP41-7, HvAP42-7, HvAP43-7, HvAP44-7 and the least chromosome number is 1 that is present in HvAP1-1, HvAP2-1 (Table 1).

3.3 Predicted gene structure and conserved motifs

It is well documented that the analysis of intron and exon structure boundaries is an important step to study the evolutionary gene relationship and functional diversification within a gene family. In the present study, by comparing the CDS and the genomic DNA sequences corresponding to individual genes, the HvAP structures were obtained Figure1. It shared the similarpatterns of exon/intron structures, including Intron phase, intron number and exon length and the conserved motifs Figure2.

3.4 Homology modeling Protein

Predicted 3D structure deduced by using automated Swiss -Model server were further visualized in CPK by UCSF CHIMERA are shown in Figure3. The calculated Ramachandran plots for the phi (Φ) and psi (Ψ) torsion angles analysis showed the excellent geometry of the modeled 3D structures of representative proteins Table 3.In procheck ,we take favoured region and percent identity above 90% and 30%. There are 6 verify 3d structures Table 4.

3.5 Phylogeny analysis

To evaluate the evolutionary relationship of 44 HvAP proteins in barley, we conducted a phylogeny analysis using MEGA 6.0 , based on full -length proteins sequences extracted from EnsemblPlants. Two clusters are formed in it that shows the phylogenetic relationship between them Figure4.

Table 1. Catalogue of identified HvAP genes in Barley genome

Gene	Gene ID	Chromosome number	Genome Location	Splice variants	bp	aa	Subcellular Localization
HvAP1-1	HORVU1Hr1G043890	1	319978255-319979652	5	1387	110	vacuole
HvAP2-1	HORVU1Hr1G047100	1	347380765-347387797	14	2060	302	cell membrane
HvAP3-2	HORVU2Hr1G010990	2	21580813-21582937	4	2125	318	cell membrane
HvAP4-2	HORVU2Hr1G013110	2	28661176-28661841	1	666	97	vacuole
HvAP5-2	HORVU2Hr1G038740	2	183517995-183520989	18	1651	341	cell membrane
HvAP6-2	HORVU2Hr1G089820	2	640711604-640714153	11	868	248	cell membrane
HvAP7-2	HORVU2Hr1G089940	2	640763978-640790201	21	3240	228	cell membrane
HvAP8-2	HORVU2Hr1G089970	2	640848559-640849918	3	1360	48	_
HvAP9-2	HORVU2Hr1G096360	2	674156049-674158319	16	1536	288	cell membrane
HvAP10-2	HORVU2Hr1G097780	2	680440771-680447491	18	1371	249	vacuole
HvAP11-3	HORVU3Hr1G001320	3	2831215-2832642	4	851	207	cell membrane
HvAP12-3	HORVU3Hr1G014440	3	33063768-33064947	4	910	58	cell membrane
HvAP13-3	HORVU3Hr1G031620	3	158035126-158036745	5	1517	251	vacuole
HvAP14-3	HORVU3Hr1G031680	3	158516891-158519111	9	1292	268	cell membrane
HvAP15-3	HORVU3Hr1G038940	3	226994907-226995260	1	354	63	vacuole
HvAP16-3	HORVU3Hr1G079560	3	584130702-584131914	7	1107	145	cell membrane
HvAP17-3	HORVU3Hr1G094900	3	648308438-648308794	1	357	118	vacuole

HvAP18-3	HORVU3Hr1G116790	3	696450874-696453390	13	1371	249	vacuole
HvAP19-4	HORVU4Hr1G024470	4	139539907-139548051	32	3524	347	peroxisome
HvAP20-4	HORVU4Hr1G052170	4	431436652-431448837	26	4083	623	cellmembrane
HvAP21-4	HORVU4Hr1G079230	4	611617220-611619921	5	2592	101	vacuole
HvAP22-4	HORVU4Hr1G085250	4	631305594-631307831	7	2238	127	vacuole
HvAP23-5	HORVU5Hr1G027240	5	156234651-156236130	5	1244	286	cell membrane
HvAP24-5	HORVU5Hr1G029550	5	178168325-178169565	7	996	255	cell membrane
HvAP25-5	HORVU5Hr1G055200	5	432818312-432819975	8	1664	232	cell membrane
HvAP26-5	HORVU5Hr1G084230	5	571502302-571503774	4	1242	311	cell membrane
HvAP27-5	HORVU5Hr1G085710	5	575459538-575465363	11	1419	333	cell membrane
HvAP28-5	HORVU5Hr1G125600	5	669246247-669247690	1	1444	291	cell membrane
HvAP29-6	HORVU6Hr1G014300	6	30371902-30373435	2	1534	292	cell membrane
HvAP30-6	HORVU6Hr1G058930	6	388366010-388370536	21	4155	121	cell membrane
HvAP31-6	HORVU6Hr1G062980	6	423193038-423194233	2	995	249	vacuole
HvAP32-6	HORVU6Hr1G064140	6	433610640-433613912	7	1356	290	cell membrane
HvAP33-6	HORVU6Hr1G075850	6	522296342-522300018	13	1582	295	cell membrane
HvAP34-6	HORVU6Hr1G092960	6	578580284-578692819	13	2222	234	cell membrane
HvAP35-6	HORVU6Hr1G092970	6	578620745-578622326	4	1371	382	chloroplast
HvAP36-7	HORVU7Hr1G038220	7	95164287-95168362	12	2493	217	cell membrane
HvAP37-7	HORVU7Hr1G038270	7	95635702-95639521	3	2177	217	cell membrane
HvAP38-7	HORVU7Hr1G038940	7	101507157-101508494	4	1139	209	cell membrane
HvAP39-7	HORVU7Hr1G043590	7	133179335-133182211	8	1326	260	cell membrane
HvAP40-7	HORVU7Hr1G043600	7	133182157-133218711	7	1191	319	cell membrane
HvAP41-7	HORVU7Hr1G072160	7	401405011-401405370	1	252	83	cell membrane

HvAP42-7	HORVU7Hr1G081770	7	488307354-488308764	10	1217	248	cell membrane
HvAP43-7	HORVU7Hr1G088900	7	539913399-539914677	1	841	211	cell membrane
HvAP44-7	HORVU7Hr1G121250	7	653463510-654284107	6	2036	286	cell membrane

Table 2. Predicted physiochemical properties of HvAP proteins

Gene	Length	Molecular weight	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity	Stable Yes/No
HvAP1-1	110	11440.6	9.84	36.89	132.18	0.885	Yes
HvAP2-1	302	31261.39	9.15	36.65	98.58	0.507	Yes
HvAP3-2	318	33385.61	9.61	48.45	88.43	0.24	No
HvAP4-2	97	10179.82	10.87	49.07	91.44	0.364	No
HvAP5-2	341	36057.91	8.94	31.66	97.95	0.478	Yes
HvAP6-2	248	undefined	undefined	33	83.83	0.392	Yes
HvAP7-2	228	23879.58	6.94	34.63	94.21	0.46	Yes
HvAP8-2	48	5291.09	6.7	25.64	95.62	0.16	Yes
HvAP9-2	288	30656.59	8.97	27.94	94.93	0.391	Yes
HvAP10-2	249	25303.32	5.9	28.38	106.63	0.854	Yes
HvAP11-3	207	22176.9	8.58	51.4	96.62	0.342	No
HvAP12-3	58	6505.59	10.51	42.84	85.69	0.243	No
HvAP13-3	251	25486.84	6.69	38.15	113.51	0.891	Yes
HvAP14-3	268	28272.02	12.3	59.86	92.2	-0.143	No
HvAP15-3	263	6639.79	8.19	18.41	99.05	0.732	Yes
HvAP16-3	145	15027.68	5.21	24.15	121.66	1.058	Yes
HvAP17-3	118	12561.54	10.83	62.75	86.69	0.199	No
HvAP18-3	252	25516.64	5.38	24.44	109.6	0.899	Yes
HvAP19-4	347	39668.65	7.75	47.58	72.31	-0.692	No
HvAP20-4	623	69289.27	5.94	41.87	74.86	-0.213	No
HvAP21-4	101	10686.37	4.9	15.32	104.26	0.637	Yes
HvAP22-4	127	13518.93	6.23	14.8	98.19	0.58	Yes
HvAP23-5	286	29782.32	7.71	27.49	101.01	0.529	Yes
HvAP24-5	255	26388.43	6.18	27.82	103.29	0.551	Yes
HvAP25-5	232	24390.86	5.63	35.21	82.41	0.214	Yes
HvAP26-5	311	32597.93	9.37	27.17	104.79	0.524	Yes
HvAP27-5	333	35343.49	8.73	30.08	91.17	0.144	Yes
HvAP28-5	291	30509.43	8.84	29.93	103.33	0.52	Yes

HvAP29-6	292	30696.34	9.1	22.2	94.69	0.334	Yes
HvAP30-6	121	12923.85	4.54	34.94	98.35	0.417	Yes
HvAP31-6	249	25205.24	5.61	23.86	107.51	0.9	Yes
HvAP32-6	290	30853.73	9	31.03	95.59	0.355	Yes
HvAP33-6	295	31660.52	6.79	39.53	99.15	0.429	Yes
HvAP34-6	234	24543.99	6.41	27.94	85.85	0.265	Yes
HvAP35-6	382	42276.35	12.12	70.92	74.58	-0.879	No
HvAP36-7	217	23191.94	6.69	30.75	97.05	0.606	Yes
HvAP37-7	217	23191.94	6.69	32.07	97.97	0.615	Yes
HvAP38-7	204	undefined	undefined	33.14	106.03	0.443	Yes
HvAP39-7	260	28489.74	6.49	45.39	87	0.218	No
HvAP40-7	319	34215.61	9.03	39.65	94.14	0.35	Yes
HvAP41-7	83	8504.08	8.99	14.68	117.83	0.759	Yes
HvAP42-7	248	25203.24	5.66	24.89	107.02	0.873	Yes
HvAP43-7	211	22707.62	7.22	33.96	72.56	-0.138	Yes
HvAP44-7	286	30522.23	5.94	28.3	95.17	0.483	Yes

Table 3. Predicted 3D structure of HvAP

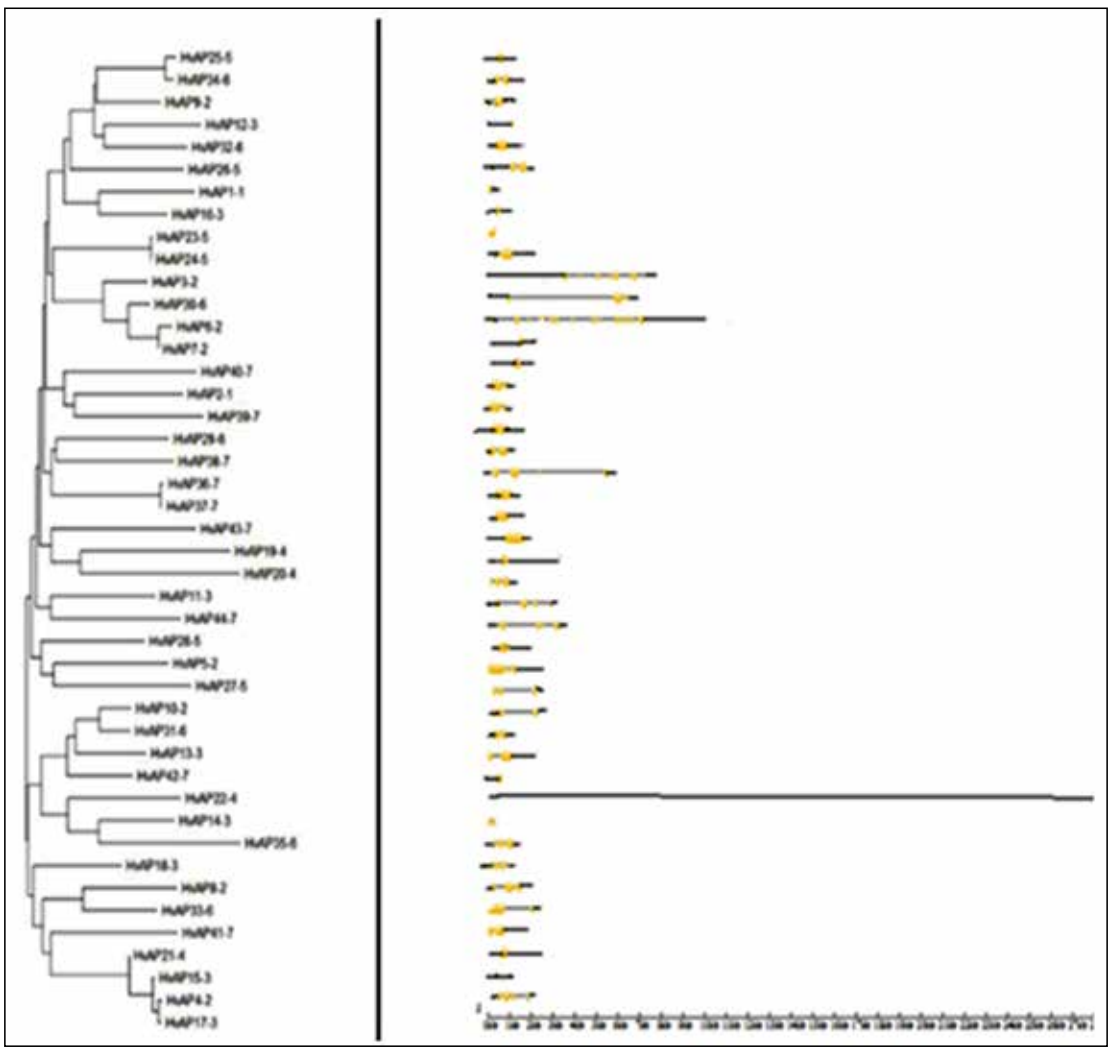
Gene	Gene ID	% Identity	Most favoured region	Additional Allowed region	Generously allowed region
HvAP1-1	HORVU1Hr1G043890	57.33%	93.50%	6.50%	0.00%
HvAP2-1	HORVU1Hr1G047100	29.46%	89.80%	8.80%	0.90%
HvAP3-2	HORVU2Hr1G010990	68.42%	91.70%	7.80%	0.50%
HvAP4-2	HORVU2Hr1G013110	52.63%	83.80%	14.70%	1.50%
HvAP5-2	HORVU2Hr1G038740	82.13%	91.60%	7.50%	0.50%
HvAP7-2	HORVU2Hr1G089940	69.70%	91.80%	7.60%	0.60%
HvAP8-2	HORVU2Hr1G089970	86.49%	83.30%	16.70%	0.00%
HvAP9-2	HORVU2Hr1G096360	71.91%	90.30%	8.70%	1.00%
HvAP10-2	HORVU2Hr1G097780	76.57%	91.00%	8.50%	0.50%
HvAP11-3	HORVU3Hr1G001320	30.28%	92.70%	4.90%	0.80%
HvAP12-3	HORVU3Hr1G014440	30.61%	77.50%	22.50%	0.00%
HvAP13-3	HORVU3Hr1G031620	48.94%	91.50%	7.40%	0.00%
HvAP14-3	HORVU3Hr1G031680	42.50%	94.90%	5.10%	0.00%
HvAP15-3	HORVU3Hr1G038940	66.67%	88.00%	12.00%	0.00%
HvAP16-3	HORVU3Hr1G079560	35.21%	90.80%	7.50%	1.70%
HvAP17-3	HORVU3Hr1G094900	47.87%	84.50%	14.10%	1.40%
HvAP18-3	HORVU3Hr1G116790	58.09%	93.00%	7.00%	0.00%

HvAP19-4	HORVU4Hr1G024470	22.22%	86.90%	11.10%	1.00%
HvAP20-4	HORVU4Hr1G052170	31.76%	85.70%	12.00%	1.10%
HvAP21-4	HORVU4Hr1G079230	68.32%	85.10%	14.90%	0.00%
HvAP22-4	HORVU4Hr1G085250	57.38%	82.90%	17.10%	0.00%
HvAP23-5	HORVU5Hr1G027240	75.09%	91.40%	8.00%	0.50%
HvAP24-5	HORVU5Hr1G029550	74.07%	93.00%	6.50%	0.50%
HvAP25-5	HORVU5Hr1G055200	73.03%	93.00%	6.50%	0.50%
HvAP26-5	HORVU5Hr1G084230	65.83%	92.50%	7.10%	0.70%
HvAP27-5	HORVU5Hr1G085710	33.48%	91.30%	8.00%	0.60%
HvAP28-5	HORVU5Hr1G125600	68.77%	89.00%	10.50%	0.50%
HvAP29-6	HORVU6Hr1G014300	67.68%	89.90%	8.70%	1.40%
HvAP30-6	HORVU6Hr1G058930	58.49%	88.90%	10.80%	0.30%
HvAP31-6	HORVU6Hr1G062980	79.18%	91.10%	8.90%	0.00%
HvAP32-6	HORVU6Hr1G064140	72.73%	90.60%	8.40%	1.00%
HvAP33-6	HORVU6Hr1G075850	32.89%	90.60%	8.40%	1.00%
HvAP34-6	HORVU6Hr1G092960	68.75%	92.00%	6.70%	1.30%
HvAP35-6	HORVU6Hr1G092970	32.00%	78.60%	21.40%	0.00%
HvAP36-7	HORVU7Hr1G038220	29.07%	91.60%	6.40%	0.70%
HvAP37-7	HORVU7Hr1G038270	30.81%	90.60%	7.40%	2.00%
HvAP39-7	HORVU7Hr1G043590	33.71%	85.00%	12.10%	2.30%
HvAP40-7	HORVU7Hr1G043600	32.88%	89.40%	9.40%	1.10%
HvAP41-7	HORVU7Hr1G072160	25.37%	76.80%	20.10%	2.70%
HvAP42-7	HORVU7Hr1G081770	71.43%	92.90%	6.60%	0.50%
HvAP43-7	HORVU7Hr1G088900	31.58%	85.40%	16.20%	2.40%
HvAP44-7	HORVU7Hr1G121250	28.84%	87.10%	10.40%	2.50%

Table 4. Predicted verify 3d structures

Gene	% Identity	Favoured region	Errat	verify 3d	prove	procheck
HvAP1-1	57.33%	93.50%	96.9811	Fail	pass	errors:1,warning:2,pass:5
HvAP3-2	68.42%	91.70%	96.3983	fail	warning	errors:3,warning:3,pass:2
HvAP5-2	82.13%	91.60%	97.7023	fail	warning	errors:4,warning:2,pass:2
HvAP7-2	69.70%	91.80%	92.5474	Fail	Warning	Errors:4,warning:2,pass:2
HvAP9-2	71.91%	90.30%	97.8947	fail	warning	errors:3,warning:3,pass:2
HvAP10-2	76.57%	91.00%	95.9866	fail	pass	errors:1,warning:5,pass:2
HvAP13-3	48.94%	91.50%	94.5143	fail	pass	errors:5,warning:2,pass:2
HVAP14-3	42.50%	94.90%	97.3034	fail	pass	errors:1,warning:3,pass:4

HvAP16-3	35.21%	90.80%	98.4283	fail	pass	errors:4,warning:2,pass:2
HvAP18-3	58.09%	93.00%	95.9322	pass	pass	errors:1,warning:4,pass:3
HvAP23-5	75.09%	91.40%	97.9778	pass	warning	errors:5,warning:2,pass:2
HvAP24-5	74.07%	93.00%	98.7443	fail	warning	errors:3,warning:3,pass:2
HvAP25-5	73.03%	93.00%	98.2172	fail	warning	errors:3,warning:3,pass:2
HvAP26-5	65.83%	92.50%	98.2172	fail	warning	errors:3,warning:3,pass:2
HvAP27-5	33.48%	91.30%	97.1282	fail	warning	errors:2,warning:4,pass:2
HvAP31-6	79.18%	91.10%	96.9061	pass	pass	errors:1,warning:4,pass:3
HvAP32-6	72.73%	90.60%	98.8248	pass	warning	errors:1,warning:5,pass:2
HvAP33-6	32.89%	90.60%	98.8248	pass	warning	errors:1,warning:5,pass:2
HvAP34-6	68.75%	92.00%	90.1639	fail	warning	errors:3,warning:4,pass:2
HvAP37-7	30.81%	90.60%	97.7456	fail	warning	errors:5,warning:1,pass:3
HvAP42-7	71.43%	92.90%	95.6624	pass	pass	errors:1,warning:2,pass:5



CDS

Figure 1. Prediction of intron/exon boundaries

Motif analysis

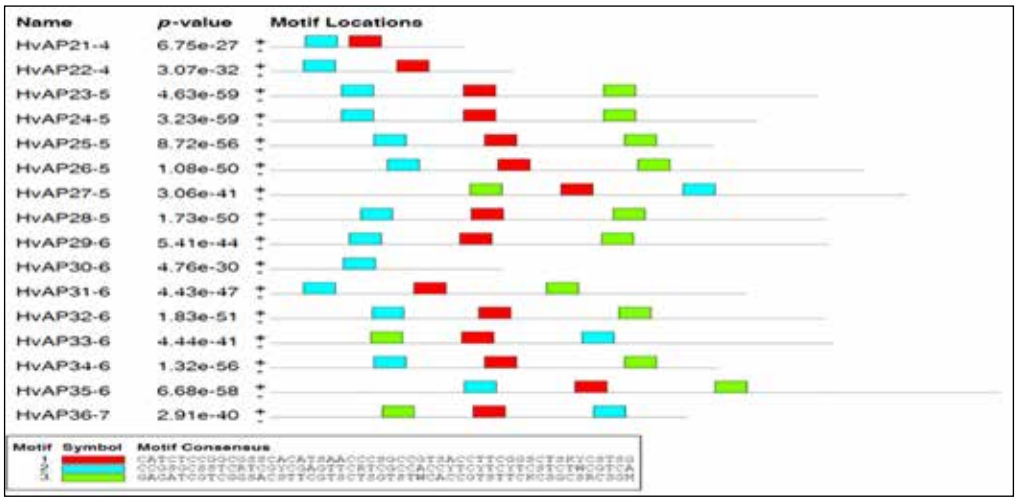
(A)

(B)

(C)

(D)

(E)



(F)

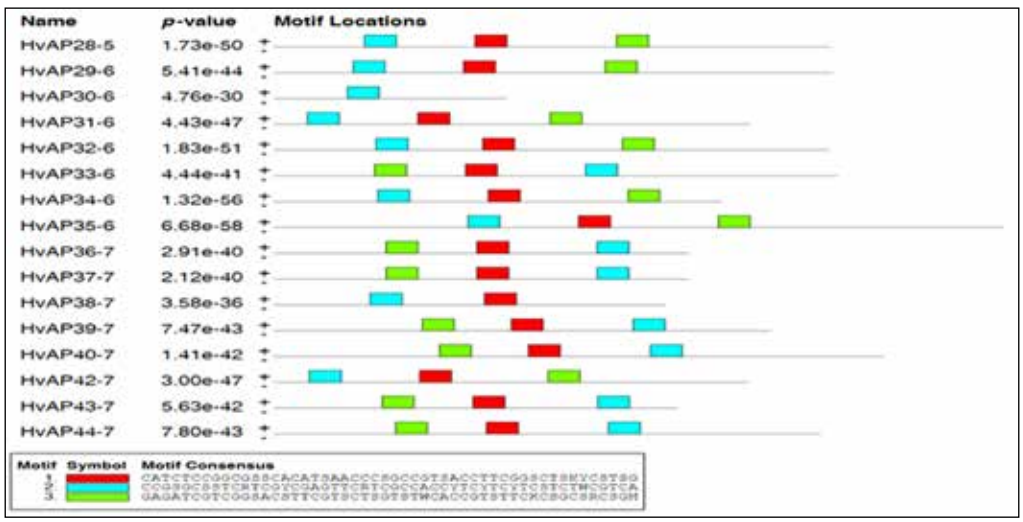
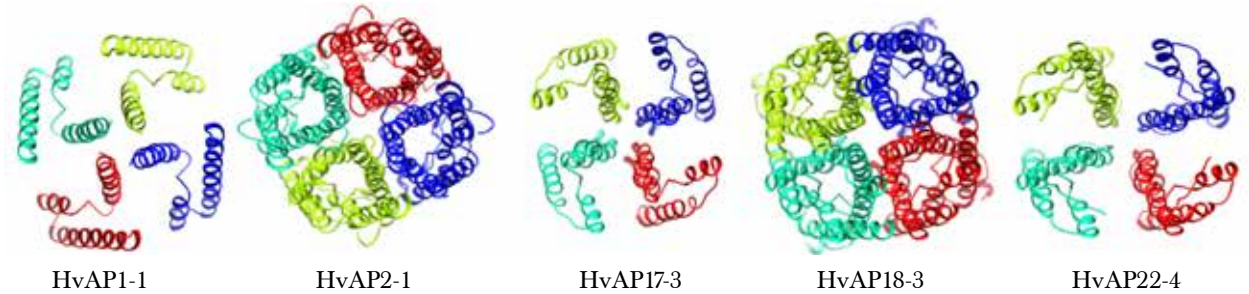


Figure 2. These shows the conserved motif location of different aquaporin genes using meme tool.



HvAP1-1 HvAP2-1 HvAP17-3 HvAP18-3 HvAP22-4

HvAP23-5 HvAP25-5 HvAP31-6 HvAP32-6 HvAP33-6

HvAP42-7 HvAP43

Figure 3. These shows the 3D structure of different aquaporin genes using swiss model.

(A)

(B)

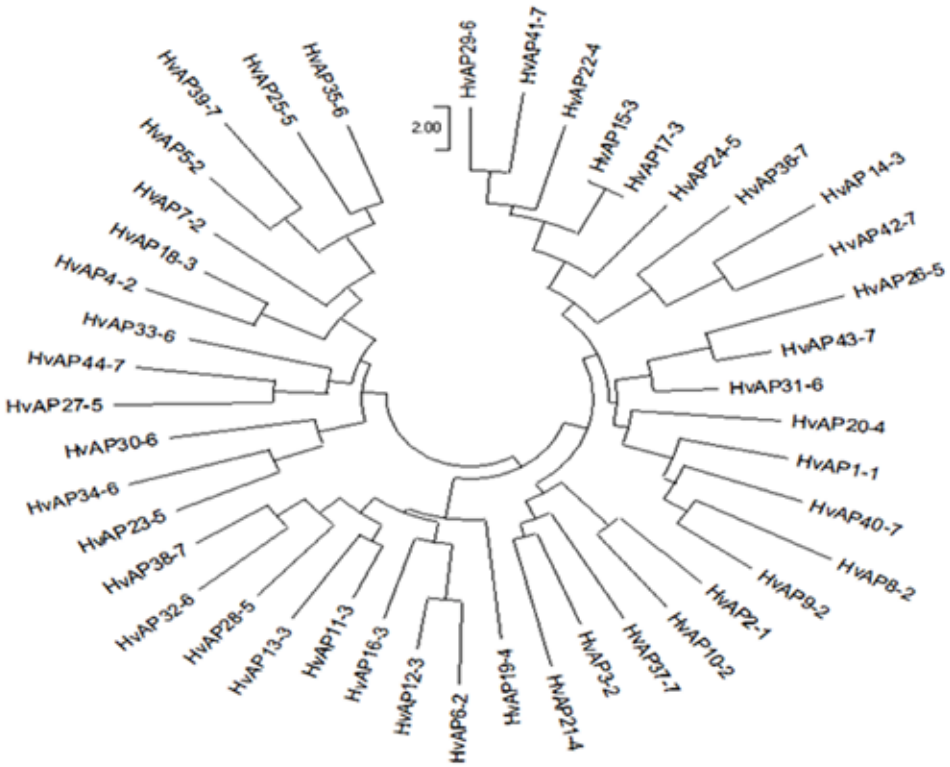


Figure 4. These shows the evolutionary relationship among Aquaporin gene in CDS and genomic sequence using mega software.

Conclusion

Using bioinformatics-based techniques and algorithms, this comprehensive genome-wide study provides a systematic identification and functional annotation of the barley aquaporin gene. Barley also known as *Hordeum vulgare*. It has been one among the earliest domesticated food crops since the origin of humanity. In terms of both area and production, barley is India's second most important winter cereal after wheat and the Aquaporins (AQPs) are membrane channel proteins found in plants and other animals that allow water to pass through plasmalemma and organelle membranes selectively and reversibly. Plant growth, development, and survival are all dependent on AQPs. There are 44 HvAP putative genes in all, out of which 21 have a sterically stable structure and in which 6 have a sterically most stable structure, indicating that they are likely to generate proteins. The classification was further validated by gene structure, sequence identity, conserved motif, and phylogeny analysis. Overall, this research gives first-hand functional and structural understanding of the aquaporin gene in barley, and the findings will assist lay the groundwork for additional functional verification of the HvAP gene during salt stress responses, resulting in barley improvement.

Author Contributions

SM and MKS prepared the manuscript and SM, SK and MKS helped in preparing the final version of the manuscript and correspond to the journal.

Ethical Approval

This article does not contain any studies involving human or animal participants performed by any of the authors.

Conflicts of Interest:

The authors declare no conflict of interest.

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