



GENOME-WIDE ANALYSIS OF CYCLIC NUCLEOTIDE-GATED ION CHANNELS (CNGCS) OF *ARABIDOPSIS THALIANA* UNDER ABIOTIC STRESSES

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SUMMARY

Cyclic nucleotide-gated ion channels (CNGCs) in plants play a significant role in abiotic and biotic stress tolerance. This study analyzed 20 CNGCs of *Arabidopsis thaliana* for their potential role under different stresses. According to phylogenetic analysis, the abiotic stress-tolerating gene CNGC19 in *A. thaliana* showed as closely related to *Hordeum vulgare* cyclic nucleotide-gated ion channel 19 (HvCNGC19) in barley and *Oryza sativa* cyclic nucleotide-gated ion channels 4 and 11 (OsCNGC4 and OsCNGC11) of rice. All CNGCs of *A. thaliana* contains an ion transport domain. HvCNGC19, OsCNGC4, OsCNGC11, and AtCNGC19 contained the same motif 24, which depicted that they might be expressed similarly to AtCNGC19 under salt stress. CNGCs expression signals under abiotic stress showed high expression of AtCNGC19 and AtCNGC20 under salt stress in roots and AtCNGC2 and AtCNGC4 in shoots, yet very low in roots under approximately all stresses. The findings provide widespread implications for future cell signaling research and characterization of CNGCs for their roles under different stresses.

Keywords: Cyclic nucleotide-gated ion channels, barley, rice, motif analysis, signaling, *Arabidopsis thaliana*

Key findings: The phylogenetic analysis of CNGCs of *A. thaliana*, rice, and barley depicted that the closely related CNGCs contain similar motifs and might be expressed similarly under different stresses. CNGCs expressed differently in roots and shoots after six and 12 hours under diverse abiotic stresses in *A. thaliana*.

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INTRODUCTION

Global food production has increased dramatically during the 20th century, with the advanced applications of irrigation, organic and chemical fertilizers, the use of agrochemicals to control weeds, pests, and pathogens, and advanced techniques in plant breeding, genetics, and bioengineering (Huang *et al.*, 2002). Proper growth and development of plants need an adequate supply of mineral nutrients. Plants, being sessile organisms, need to develop multiple transport pathways and significant elasticity in their uptake capacity to adjust to the specific environment and tissue requirements (Gobert *et al.*, 2006). The reproductive phase of flowering plants can be highly sensitive to the stresses of hot or cold temperatures. A single hot day or cold night can sometimes be fatal to reproductive success (Zinn *et al.*, 2010).

Salinity and drought are two environmental factors determining plant productivity and distribution. The average yields for most major crop plants are declining on a global scale by more than 50% due to desertification and salinization, and more than 10% of arable lands, as well (Egamberdieva *et al.*, 2019). Drought affects crops in many ways, and identification of drought-tolerant genetic resources and figuring out the best method to prevent crop loss are therefore crucial (Ahmad *et al.*, 2022). Plants recognize abiotic stresses, which respond appropriately by altering their growth, development, and metabolism (Ahmad *et al.*, 2021). The regulatory circuits and signaling pathways, comprised of a network of proteins, transcription factors, promoters, and output proteins and metabolites, all include the stress sensors (Bartels and Sunkar, 2005).

For sustainable and long-term global food security, agricultural plants more resistant to abiotic challenges like salinity and drought need further development (Shahzad *et al.*, 2021). This kind of investigation requires a complete genomic sequence in the public domain, quick generation times, expressed sequence tags (EST), microarray and proteomics data, and, ideally, a large set of well-characterized mutants. Many research regarded *Arabidopsis* as the best model system to study water and salt stress at the molecular and system levels (Denby and Gehring, 2005).

The signaling molecules cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are essential for the function of the CNGC genes in *A. thaliana*. Specific stress reactions brought

on by cAMP lead to modifications in cellular energy homeostasis (Alqurashi *et al.*, 2016). The cNMPs, 3', 5'-cGMP, and cAMP primarily control prokaryotes and eukaryotes' varied cellular functions, including responses to biotic and abiotic stressors (Jammes *et al.*, 2011). The direct and reversible binding of cAMP and cGMP to the cyclic nucleotide-binding domain (CNBD) activate plant CNGCs. Cyclic nucleotides cannot bind to CNBD in the presence of calcium, and CNGCs remain inactive (Kohler and Neuhaus, 2000). These ligand-gated calcium-permeable channels commonly occur in the plasma membrane, where their efficient activation comes from cyclic nucleotide having high affinity and intricately controlled by calmodulin (CaM) binding to the CaM binding domain (Ma *et al.*, 2007; Wang *et al.*, 2013; Gao *et al.*, 2014; Zhang *et al.*, 2018). These signaling molecules have a variety of involvement in physiological processes, including photomorphogenesis, salt stress tolerance, gibberellic acid-induced signaling in barley, and phytochrome signaling (Nawaz *et al.*, 2014).

CNGCs were first identified as non-selective cation channels in olfactory sensory neurons and retinal photoreceptors (Kaupp and Seifert, 2002), with plant CNGCs recognized first in barley (Bridges *et al.*, 2005). The function of CNGCs displays the prevention of the sodium ion distribution in the protoplast of *A. thaliana*. The CNGCs are involved in salinity tolerance and can control Na⁺ uptake (Bridges *et al.*, 2005). CNGCs have signaling pathways leading to hypersensitivity response (HR) resistance. They also act as a positive regulator of resistance against fungal pathogens. Reports disclosed that 15 flowering plants contained 333 CNGC proteins, of which 18 were from tomatoes, 20 from *Arabidopsis* (Maser *et al.*, 2001), 28 from rice, five from *Selaginella moellendorffii*, and eight from *Physcomitrella patens* (Zelman *et al.*, 2012). The most crucial channels for conducting calcium ions in signal transduction are cyclic nucleotide-gated ion channels (CNGCs) (Talke *et al.*, 2003). CNGCs, which have 20 members in *Arabidopsis* and 18 members in the tomato, are highly conserved across plant species (Saand *et al.*, 2015). CNGCs linked cyclic nucleotide and calcium signals. The huge gene family that codes for the calcium-dependent kinases (CDPKs) have a role in their ability to withstand environmental stressors like cold, salinity, and drought (Talke *et al.*, 2003).

This study aimed to characterize the CNGC family of *A. thaliana* and their expression analysis under different abiotic stresses. The

use of bioinformatics tools performed a phylogenetic and motif analysis among *A. thaliana*, rice, and barley CNGCs to discover functional similarities.

MATERIALS AND METHODS

Phylogenetic relationship of *Arabidopsis*, rice, and barley CNGCs

The amino acid sequences of 20 CNGCs came from The Arabidopsis Information Resource (TAIR), then aligned using the Clustal W algorithm of the Molecular Genetic Analysis (MEGA version 6.0) software platform. Determining the phylogenetic link between *A. thaliana*, barley, and rice resulted from constructing a phylogenetic tree using the database, Surveyed conserved motif Alignment diagram, and the Associating Dendrogram (SALAD) <http://salad.dna.affrc.go.jp/salad/en/>. Using the "Barley gene expression database" (beX-db) (<http://barleyflc.dna.affrc.go.jp/bexdb/>) and the "Rice expression profile" database (RiceXPro) (<http://ricexpro.dna.affrc.go.jp/>), respectively, retrieved the amino acid sequences of the CNGCs of barley and rice.

Protein analysis of *Arabidopsis* CNGCs

Using the amino acid sequences of 20 CNGCs of *A. thaliana*, analyzed their domains and motifs. Employing the database Pfam (<http://pfam.xfam.org/search/sequence>) detected the number of motifs, while the Simple Modular Architecture Research Tool (SMART) database (<http://smart.embl-heidelberg.de/>) constructed the structures of domains and motifs. This research analyzing motifs of *A. thaliana*, barley, and rice used the database SALAD (<http://salad.dna.affrc.go.jp/salad/en/>).

Genome-wide expression of CNGCs under abiotic stresses

Employing nine abiotic stresses, including cold, osmotic, salt, drought, genotoxic, oxidative, UV-B, wounding, and heat stresses, evaluated the expression of five groups of CNGCs of *A. thaliana*. The CNGCs expression data were extracted from the database Arabidopsis eFP Browser (<http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?primaryGene>) after six and 12 hours of stress in shoots and roots.

RESULTS

Evolutionary relationship of *A. thaliana*, barley, and rice CNGCs

The phylogenetic relationship of CNGCs of *A. thaliana* with barley and rice CNGCs (Figure 1) showed that only two CNGCs, AtCNGC11 and AtCNGC12, were located on smaller clad A2; HvcCNGC7 and HvcCNGC5 showed closely located, while OsCNGC17 was on the separate branch on the same clad A1aIi. The OsCNGC6 and HvcCNGC9, AtCNGC7 and AtCNGC8, and AtCNGC6 and AtCNGC9 were placed on sub-clads, while HvcCNGC8 and AtCNGC5 were located on separate branches of the same clad A1aIii. Similarly, HvcCNGC1 and OsCNGC12 were on sub-clad and AtCNGC1 on a separate branch of the clad A1aIii. Likewise, AtCNGC10 and AtCNGC13 were on sub-clad, with AtCNGC3 located on a separate branch of the clad A1aIiii. The A1bI carried only two CNGCs, HvcCNGC17 and OsCNGC15. AtCNGC17 and OsCNGC5 were on the same sub-clad while AtCNGC14 was on a separate branch; likewise, AtCNGC18 and AtCNGC16 were on the same sub-clad while HvcCNGC 18 was on a separate branch of the clad A1bIii. OsCNGC3 and AtCNGC15 were on the clad of A1bIiii. The AtCNGC19 and AtCNGC20 were located on the same clad B1a, while HvcCNGC19 and OsCNGC4 were on the same sub-clad, with OsCNGC11 on a separate branch of the clad B1b. OsCNGC7 and AtCNGC2 appeared on sub-clad B2a, while HvcCNGC15, OsCNGC1, OsCNGC9, and HvcCNGC14 emerged on the branches of the same clad, with AtCNGC4 located on a separate branch of the sub-clad of B2b.

Protein analysis of *Arabidopsis* CNGCs

All 20 CNGCs of *A. thaliana* carried ion transport domains. The CNGCs of group I, AtCNGCs (1, 3, 10, 11, 12, and 13) carried a large ion transport domain at different positions on CNGCs comprised of 319 to 325 amino acids (Figure 2). CNGCs of group II, AtCNGCs (5, 6, 7, 8, and 9), contained an ion transport domain comprised of 323 to 329 amino acids and a cNMP domain comprising 87 to 89 amino acids. AtCNGC6 and AtCNGC9 carried an ion trans_2 domain within the ion transport domain composed of 63 and 64 amino acids, respectively. All CNGCs of group II, except AtCNGC7, carried IQ calmodulin

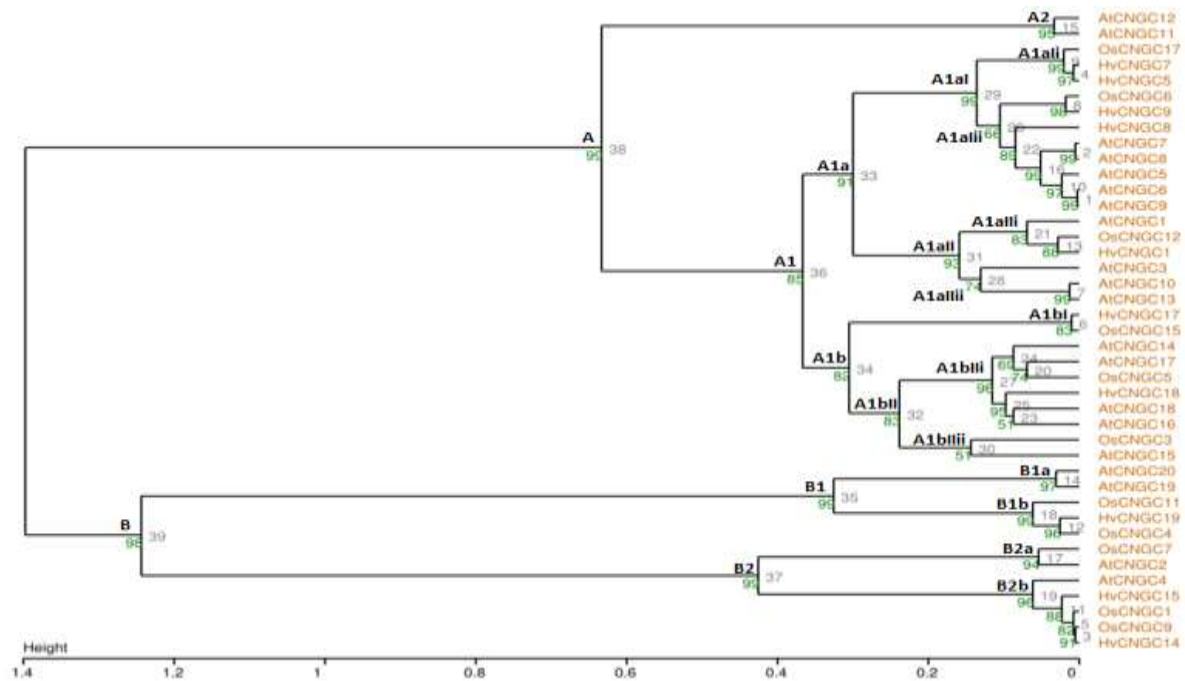


Figure 1. Phylogenetic tree of CNGCs of *A. thaliana*, barley, and rice. The amino acid sequences of *A. thaliana*, barley, and rice were aligned by the SALAD database (<http://salad.dna.affrc.go.jp/salad/en/>). It shows that *OsCNGC4*, *OsCNGC11* of rice and *HvCNGC19* of barley are present on the same clad of CNGC19 and CNGC20 of *A. thaliana* depicting that the CNGCs of rice and barley might have the same function as CNGC19 and CNGC20 in *A. thaliana*.

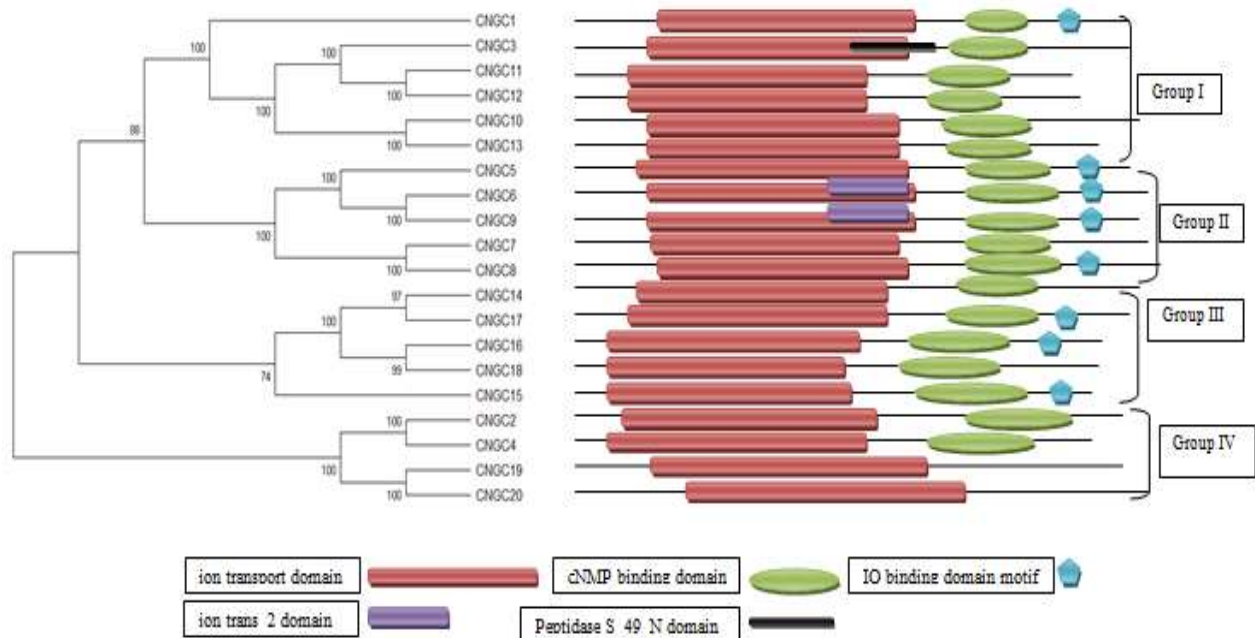


Figure 2. Domains and motifs present on 20 CNGCs of *A. thaliana*. The amino acid sequences of CNGCs were used for domains and motif analysis by using the SMART database (<http://smart.embl-heidelberg.de/>). Except for CNGC19 and CNGC20, all CNGCs contained additional domain and motifs other than ion transport domains. Ion transport domain in CNGCs are responsible for the transportation of materials.

Table 1. Domain analysis of CNGCs.

CNGCs	No. of Motifs	Pfam	Position(Independent E-value)	Description
1	3	Ion_trans	94..413(2.1e-33)	PF00520, Ion transport protein
		cNMP_binding	507..596(1.8e-08)	PF00027, Cyclic nucleotide-binding domain
		IQ	624..639(0.12)	PF00612, IQ calmodulin-binding motif
2	2	Ion_trans	125..449(3e-10)	PF00520, Ion transport protein
		cNMP_binding	550..641(6e-08)	PF00027, Cyclic nucleotide-binding domain
3	3	Ion_trans	81..405(2.2e-51)	PF00520, Ion transport protein
		cNMP_binding	498..586(0.00028)	PF00027, Cyclic nucleotide-binding domain
		Peptidase_S49_N	375..445(0.65)	PF08496,Peptidase family S49 N-terminal
4	2	Ion_trans	90..413(2.5e-10)	PF00520, Ion transport protein
		cNMP_binding	517..604(1.1e-05)	PF00027, Cyclic nucleotide-binding domain
5	3	Ion_trans	98..424(1.7e-33)	PF00520, Ion transport protein
		cNMP_binding	520..608(9.8e-08)	PF00027, Cyclic nucleotide-binding domain
		IQ	636..653(0.31)	PF00612, IQ calmodulin-binding motif
6	4	Ion_trans	115..440(8.1e-32)	PF00520, Ion transport protein
		cNMP_binding	536..623(2.4e-06)	PF00027, Cyclic nucleotide-binding domain
		IQ	652..670(0.059)	PF00612, IQ calmodulin-binding motif
		Ion_trans_2	372..436(0.16)	PF07885, Ion channel
7	2	Ion_trans	100..429(5.7e-32)	PF00520, Ion transport protein
		cNMP_binding	524..612(2.3e-08)	PF00027, Cyclic nucleotide-binding domain
8	3	Ion_trans	107..435(6.5e-31)	PF00520, Ion transport protein
		cNMP_binding	529..618(2.9e-08)	PF00027, Cyclic nucleotide-binding domain
		IQ	646..662(0.096)	PF00612, IQ calmodulin-binding motif
9	4	Ion_trans	116..439(9.7e-34)	PF00520, Ion transport protein
		cNMP_binding	535..622(6.6e-07)	PF00027, Cyclic nucleotide-binding domain
		IQ	651..669(0.0076)	PF00612, IQ calmodulin-binding motif
		Ion_trans_2	372..435(0.054)	PF00612, IQ calmodulin-binding motif
10	2	Ion_trans	77..401(7.9e-40)	PF00520, Ion transport protein
		cNMP_binding	493..587(1.1e-06)	PF00027, Cyclic nucleotide-binding domain
11	2	Ion_trans	40..363(1.2e-24)	PF00520, Ion transport protein
		cNMP_binding	454..544(6.1e-05)	PF00027, Cyclic nucleotide-binding domain
12	2	Ion_trans	40..363(1e-26)	PF00520, Ion transport protein
		cNMP_binding	456..529(2.4e-06)	PF00027, Cyclic nucleotide-binding domain
13	2	Ion_trans	77..402(7.6e-40)	PF00520, Ion transport protein
		cNMP_binding	495..584(1.7e-06)	PF00027, Cyclic nucleotide-binding domain
14	2	Ion_trans	83..408(1.8e-30)	PF00520, Ion transport protein
		cNMP_binding	504..591(1.8e-08)	PF00027, Cyclic nucleotide-binding domain
15	3	Ion_trans	79..398(1.1e-28)	PF00520, Ion transport protein
		cNMP_binding	494..582(4.2e-07)	PF00027, Cyclic nucleotide-binding domain
		IQ	609..626(0.19)	PF00612, IQ calmodulin-binding motif
16	3	Ion_trans	55..384(1.4e-29)	PF00520, Ion transport protein
		cNMP_binding	480..567(2.7e-08)	PF00027, Cyclic nucleotide-binding domain
		IQ	599..611(0.27)	PF00612, IQ calmodulin-binding motif
17	3	Ion_trans	82..408(2.4e-28)	PF00520, Ion transport protein
		cNMP_binding	504..591(5.9e-07)	PF00027, Cyclic nucleotide-binding domain
		IQ	619..634(0.43)	PF00612, IQ calmodulin-binding motif
18	2	Ion_trans	49..377(2.1e-24)	PF00520, Ion transport protein
		cNMP_binding	472..559(5.3e-10)	PF00027, Cyclic nucleotide-binding domain
19	1	Ion_trans	172..489(2.5e-21)	PF00520, Ion transport protein
20	1	Ion_trans	204..519(2.3e-25)	PF00520, Ion transport protein

motif domain of short sequence of 16 to 18 amino acids, and CNGCs of group III (14, 15, 16, 17, and 18) carried ion transport domain of 319 to 328 amino acids and cNMP domain of 87 to 88 amino acids. CNGC19 and CNGC20 of group IVa carried only ion transport domains of 317 and 315 amino acids, respectively, while CNGC2 and CNGC4 of group IVb carried ion transport domains of 324 and 317 amino acids,

as well as, cNMP binding domains of 91 and 87 amino acids, respectively (Table 1).

Another database, SALAD, separately analyzed the motifs of *A. thaliana*, barley, and rice (Figure 3). Some motifs like 1, 2, 3, 7, 9, 10, 11, 13, and 15 are common in all CNGCs of *A. thaliana*, barley, and rice, but motif 24 is only present in *AtCNGC19*, *AtCNGC20*, *OsCNGC11*, and *HvCNGC19*, showing that

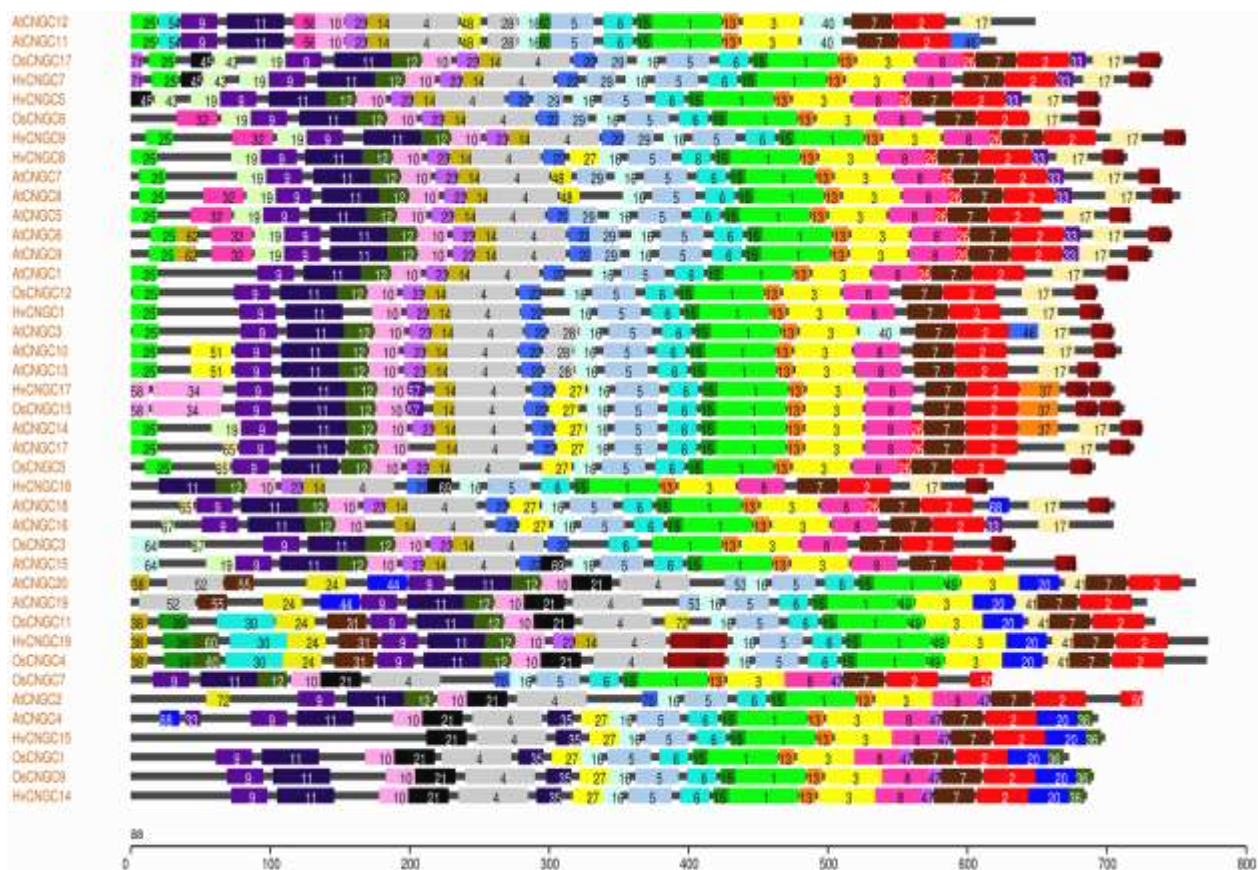


Figure 3. Motifs analysis of amino acid sequences of CNGCs of *A. thaliana*, barley, and rice was performed with the help of the SALAD database (<http://salad.dna.affrc.go.jp/salad/en/>). The conserved motifs of CNGCs of *A. thaliana*, *Hordeum vulgare*, and *Oryza sativa* represent their structural and functional similarities.

these CNGCs might have similar expression under salt stress as *AtCNGC19* expresses, and *AtCNGC2*, *OsCNGC4*, and *OsCNGC7* have a common motif 72. Motif number 30 was only present in *OsCNGC11*, *HvCNGC19*, and *OsCNGC4*, with motif number 64 present only in *OsCNGC3* and *AtCNGC15* and motif 44 in *AtCNGC19* and *AtCNGC20*.

Whole genome expression of CNGCs under abiotic stresses

Expression of group I AtCNGCs

The *AtCNGCs* expression has been shown group-wise in graphs. In group I, all six *AtCNGCs* (1, 3, 10, 11, 12, and 13) appeared highly expressed in shoots under UV-B stress, except *AtCNGC12* displaying high expression in roots under salt stress after 6 h. Yet, the maximum expression emerged in *AtCNGC3* and *AtCNGC11* under both of these stresses (Figure

4). *AtCNGC1* showed higher expression under salt stress after 12 h, with the expression of the rest of *AtCNGCs* under all abiotic stresses showing very low.

Expression of group II CNGCs

The expression of *AtCNGC5* and *AtCNGC6* in roots exhibited high under osmotic, drought, oxidative, UV-B, and wounding stresses after 6 h, but the expression of both *AtCNGCs* showed low under cold, salt, genotoxic, and heat stresses. The expression of *AtCNGC5* was higher under osmotic, drought, oxidative, UV-B, and heat stresses and low under cold, salt, genotoxic, and wounding in shoots after 12 h, with the *AtCNGC6* expression higher under osmotic stress and slightly higher under genotoxic and oxidative stresses, but lower under cold, drought, UV-B, salt, wounding, and heat stresses in shoots after 12 h (Figure 5).

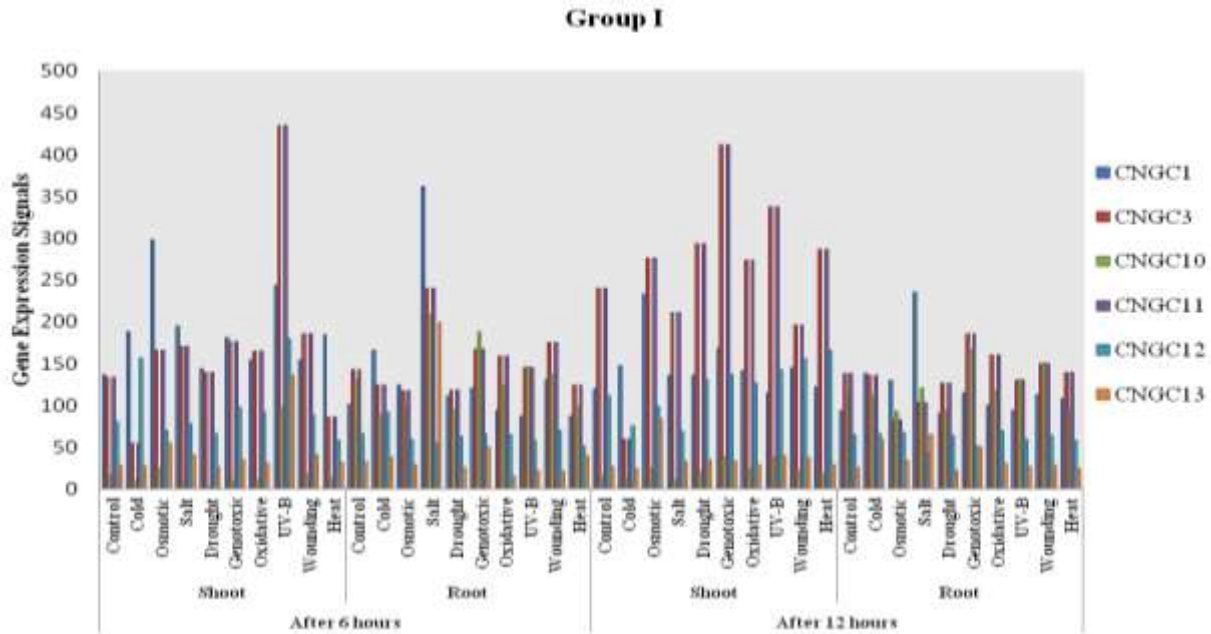


Figure 4. Group I CNGCs (CNGC1, CNGC3, CNGC10, CNGC11, CNGC12, and CNGC13) expression under abiotic stresses. The Gene Chip Operating Software (GCOS) was used for the calculation of the expression values with the specified parameters: TGT = 100 and Bkg = 20. The X-axis showed the nine abiotic stresses after 6 and 12 h and gene expression signals were shown along the Y-axis. The expression of CNGC3 and CNGC11 was similar under stresses in the root and shoot after 6 and 12 h and the expression of CNGC10 was lower in the root and higher in the shoot.

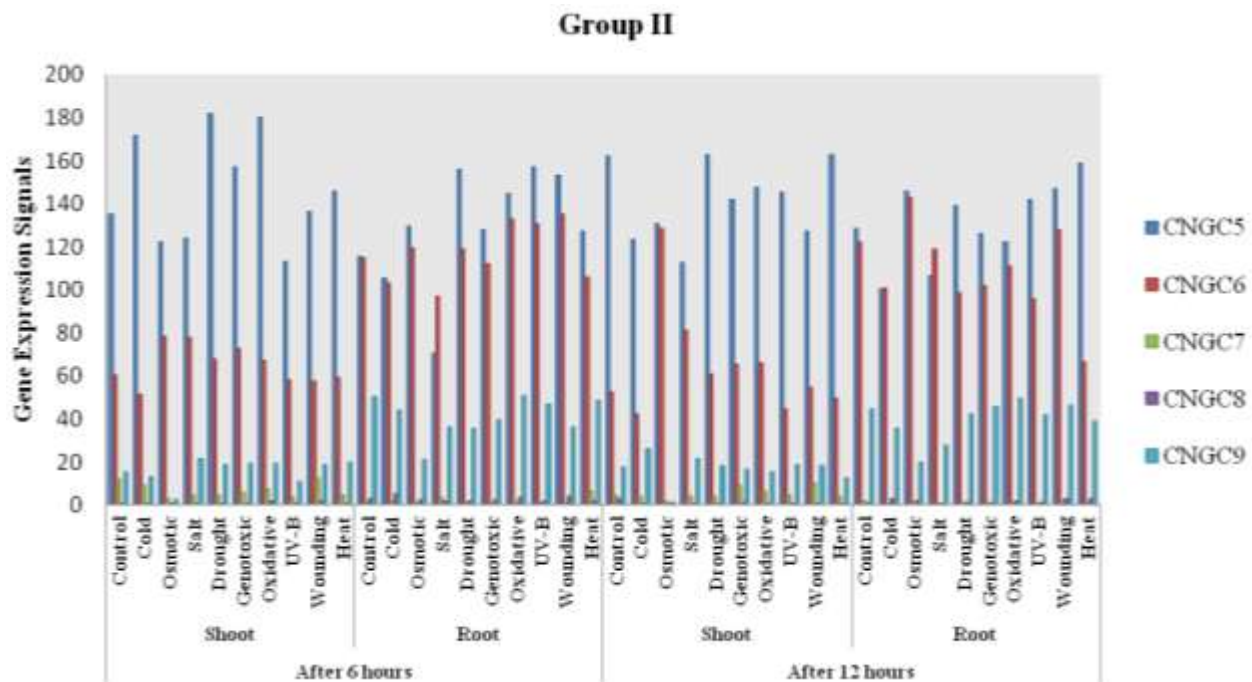


Figure 5. Group II CNGCs (CNGC5, CNGC6, CNGC7, CNGC8, and CNGC9) expression under abiotic stress. The expression of CNGC5 was remarkable as compared with other CNGCs of the same group and CNGC8 showed very low expression in root and shoot after 6 and 12 h.

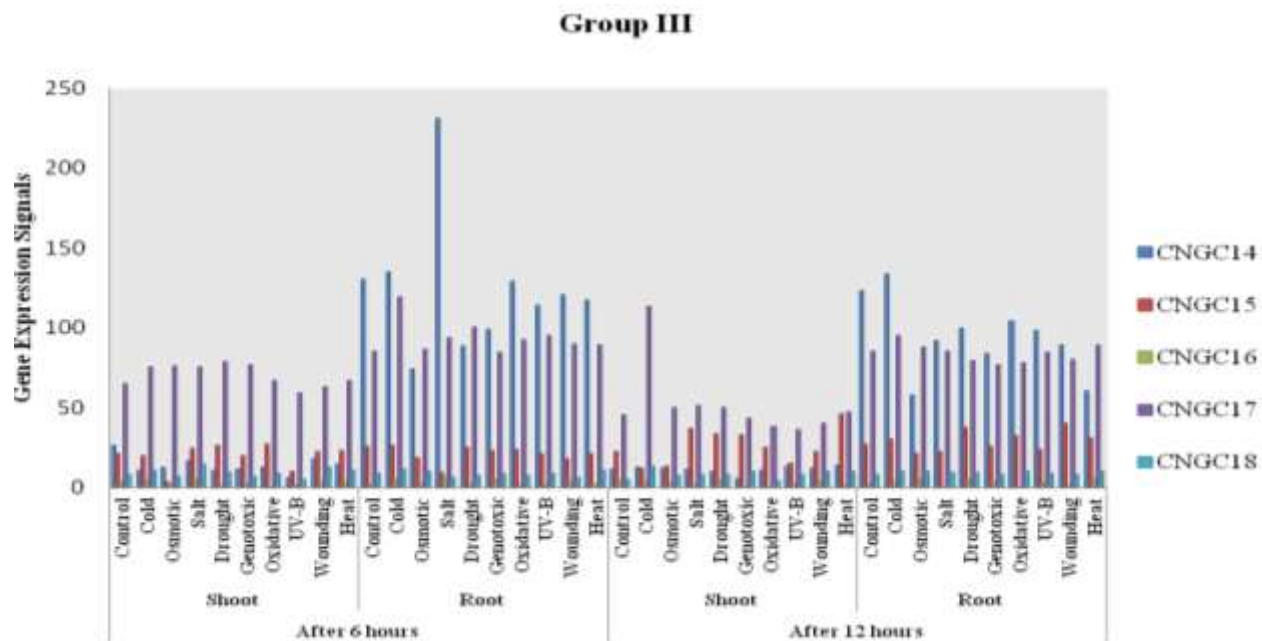


Figure 6. Group III CNGCs (CNGC14, CNGC15, CNGC16, CNGC17, and CNGC18) expression under abiotic stresses. Expression of CNGC14 was lower in the shoot and higher in the root and expression of CNGC16 and CNGC18 was approximately similar in the root and shoot after 6 and 12 h.

The *AtCNGC6* expression exhibited higher under osmotic, salt, oxidative, and wounding stresses, though lower under cold, drought, genotoxic, UV-B, and heat stresses in roots after 12 h. The *AtCNGC9* showed very slight expression under wounding and genotoxic stresses in shoots and roots after 6 and 12 h, with no or less expression under all other stresses. The expression of *AtCNGC8* also bared very low or inconsiderable in shoots and roots under all these nine abiotic stresses. The overall maximum expression appeared with *AtCNGC5* of this group.

Expression of group III *AtCNGCs*

The expression of *AtCNGCs* (14, 16, and 18) indicated very low under all nine stresses in shoots after 6 h, whereas *AtCNGC17* showed slightly higher and almost similar expression in shoots and roots after 6 and 12 h. *AtCNGC14* showed maximum expression under salt stress after 6 h in roots and was slightly higher under the rest of the stresses after 6 h in roots. The *AtCNGC14* showed significantly higher expression in shoots under all nine stresses after 12 h, though it showed slightly lower expression under osmotic, genotoxic, and heat stresses. The expression of *AtCNGC16* and *AtCNGC18* displayed inconsiderably less under

all stresses in shoots and roots after 12 h (Figure 6).

Expression of group IVA *AtCNGCs*

An inconsiderable expression of *AtCNGC19* in shoots under all stresses occurred after 6 h. The *AtCNGC20* expression was slightly higher under osmotic and UV-B stresses but was lower under the rest of the stresses in shoots after 6 h. The expression of *AtCNGC19* and *AtCNGC20* showed very high under salt stress in roots after 6 h. Indeed, the expression of *AtCNGC19* gave maximum compared with the whole family of *AtCNGCs*. *AtCNGC19* again showed slightly higher expression under salt stress in roots after 12 h, whereas it showed very low expression under all the rest of the stresses in shoots and roots after 12 h. The expression of *AtCNGC20* was slightly higher under osmotic stress in shoots and under salt stress in roots after 12 h but showing very little expression under the rest of the stresses in shoots and roots after 12 h (Figure 7).

Expression of group IVB *AtCNGCs*

In group IVb, the expression of *AtCNGC2* was very low under cold and UV-B stresses in shoots yet maximum under salt and heat

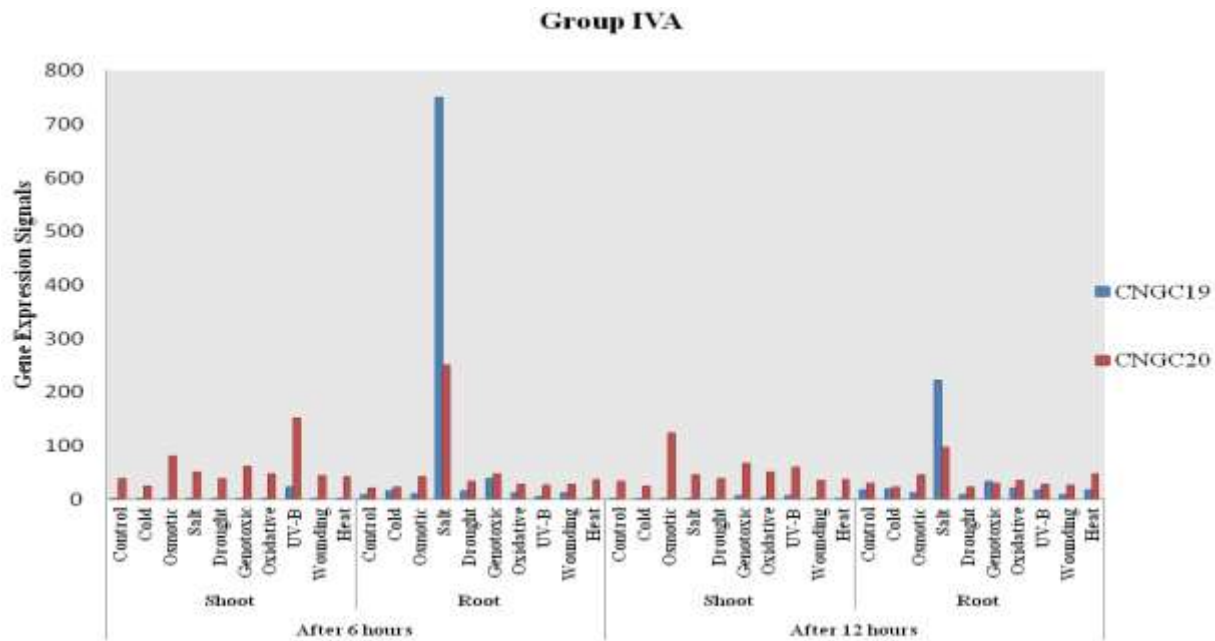


Figure 7. Group IVA CNGC19 and CNGC20 expression under abiotic stresses. It indicated that expression of CNGC19 and CNGC20 was higher in roots under salt stress as compared with other stresses after 6 and 12 h.

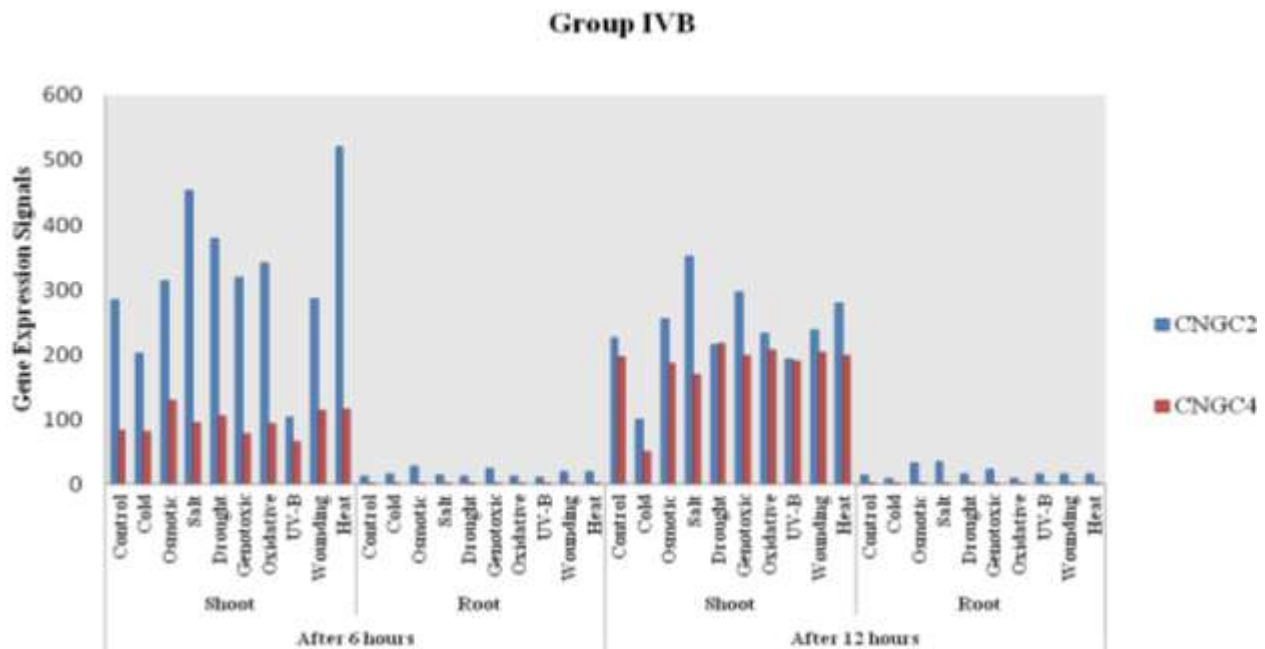


Figure 8. Group IVB CNGC2 and CNGC4 expression under abiotic stresses. It indicated that expression of both CNGCs is higher in shoots and no expression in roots after 6 and 12 h duration.

stresses and slightly higher under cold, osmotic, genotoxic, osmotic, and wounding stresses in shoots after 6 h. *AtCNGC4* showed slightly higher and almost similar expressions under all nine stresses in shoots after 6 h. Both

of these *AtCNGCs* showed less expression under all these stresses in roots after 6 h. The expression of *AtCNGC2* was higher under salt, genotoxic, and heat stresses in shoots and slightly higher under osmotic, oxidative, and

wounding stresses but low under cold, drought, and UV-B stresses in shoots after 12 h. Meantime, the *AtCNGC4* expression was lower under cold stress in shoots, with similar expressions under the rest of the stresses after 12 h. The expression of *AtCNGC2* and *AtCNGC4* showed inconsiderably in roots under all stresses after 12 h (Figure 8).

DISCUSSION

The 20 CNGCs in *A. thaliana* got divided into five groups (I, II, III, IVA, and IVB). Group I carries CNGCs (1, 3, 10, 11, 12, and 13), Group II CNGCs (5, 6, 7, 8, and 9), Group III (14, 15, 16, 17, and 18), Group IVA CNGCs (19 and 20), and Group IVB CNGCs (2 and 4) (Maser *et al.*, 2001). CNGCs play vital functions in signaling mechanisms, plant development, and abiotic stress, especially salinity, drought, cold, and biotic stress (Tunc-Ozdemir *et al.*, 2013). As an illustration, *AtCNGC1* participates in calcium uptake (Ma *et al.*, 2007), and *AtCNGC2* and *AtCNGC4* closely relate to each other by being present in the same group IVb. Both contribute to calcium ion import, defense response, and nitric oxide-mediated signal transduction (Kaplan *et al.*, 2007). Studies discovered that *AtCNGC2*, *AtCNGC4*, *AtCNGC11*, and *AtCNGC12*, with their homologs, are crucial for disease resistance to infections (Jurkowski *et al.*, 2004; Saand *et al.*, 2015; Zhang *et al.*, 2018). According to a study, *HvCNGC4* and *AtCNGC4* share 67% of their amino acid sequences, which may indicate that a mutation in the *nec1* gene comparable to *dnd2* in *A. thaliana* affects barley disease resistance (Rostoks *et al.* 2006).

AtCNGC6 aids in the uptake of Ca^{2+} and hence mediates the development of thermotolerance (Gao *et al.*, 2014). *AtCNGC16* and *AtCNGC18* are essential for pollen fertility under stress, and pollen tip growth, respectively, with *AtCNGC19* and *AtCNGC20* showing a link to salt tolerance (Frietsch *et al.*, 2007; Kugler *et al.*, 2009). *AtCNGC19* and *AtCNGC20* appeared significantly expressed after six hours of salt stress before decreasing after 12 hours (Oranab *et al.*, 2021).

The phylogenetic relationship of CNGCs of *A. thaliana*, barley, and rice showed that they are closely related to each other, like *AtCNGC1*, *HvCNGC1*, and *OsCNGC12* emerging on the same clad, which showed that they might have the same function of calcium uptake and regulation of membrane potential as CNGC1 in *A. thaliana* (Ma *et al.*, 2007). *AtCNGC19* is very close to *HvCNGC19*,

OsCNGC4, and *OsCNGC11*. *AtCNGC19* contributes to membrane potential and transcription regulation and relates to salt tolerance (Kugler *et al.*, 2009). Thus, *HvCNGC19*, *OsCNGC4*, and *OsCNGC11* might be expressed highly under salinity stress. *AtCNGC2* is closely related to *OsCNGC7*, and *AtCNGC2* participates in the import of calcium ions, defense response, nitric oxide-mediated signal transduction, plant-type hypersensitive response, and regulation of membrane potential (Kaplan *et al.*, 2007). *OsCNGC7* might be expressed in the same way in rice as *AtCNGC2* in *A. thaliana*. *AtCNGC4* is closely related to *HvCNGC15* and *HvCNGC14*. *AtCNGC4* also engages in hypersensitive response and regulation of membrane potential (Kaplan *et al.*, 2007). Fluxes of Ca^{2+} and K^{+} are among the earliest events possibly detected in defense signaling; however, it is unclear how defensive responses work or how the ion channel mediates the HR (hypersensitive reaction) (Blume *et al.*, 2000). After the initiation of signal transduction pathways caused by the identification of stress stimulus, secondary messengers, such as Ca^{2+} , are produced (De-Silva *et al.*, 2011). Ca^{2+} and K^{+} influxes can result from mediation by an ion channel formed by *AtCNGC2*, but a significant Na^{+} influx is not permitted (Leng *et al.*, 2002).

The presence of the same motifs on CNGCs in *A. thaliana*, barley, and rice depicted that they might have the same function in plasma membrane regulation and signaling mechanisms. This motif analysis helped to understand that there were some specific motifs on the particular CNGCs, as motif 24 is present only in *AtCNGC19*, *AtCNGC20*, *OsCNGC11*, *HvCNGC19*, and *OsCNGC4*. These CNGCs might express high salinity stress, as seen in *AtCNGC19*. The presence of common motif 72 on *AtCNGC2* and *OsCNGC7* made them related to each other in a function, like a defense mechanism. A complex network of interactions among phytohormones, microRNAs, and protein-coding genes regulates several plant developmental processes. Phytohormones and miRNAs are crucial regulators for the plant's proper growth and development in both ideal and stressful conditions by controlling gene expression (Ahmad *et al.*, 2022).

Numerous relevant studies have offered convincing evidence that specific physiological functions and molecular traits of non-selective cation channels (NSCCs), like CNGCs, directly relate to a plant's variety of stress-adaptive responses, growth and development, acquisition of nutrients, and

other physiological processes (Hasanuzzaman et al., 2018). The abnormally high levels of exogenous calcium may have exacerbated the abnormalities in *AtCNGC2*, which impact both vegetative and reproductive development (Chan et al., 2003). Identification of the functions of these genes under various circumstances would highly benefit the genetic engineering of plants facing abiotic and biotic stresses.

CONCLUSIONS

The phylogenetic relationship and presence of the same motifs on CNGCs of barley, rice, and *Arabidopsis* depicted that they evolved from the common ancestral genes, suggesting having similar functions and expressions under stress conditions. Some CNGCs are well characterized, like *AtCNGCs* (2, 4, 16, 18, 19, and 20), with the rest still for complete characterization. The remaining CNGCs also need further exploring for their potential role under different conditions. CRISPR/Cas9, a genome editing technique, is an advanced and efficient technique that can study knock-out CNGCs in plants and could prove very helpful for the efficiencies of CNGCs under different stress conditions. The signaling mechanism of all CNGCs still seeks full explanation. More study and research activities require action to understand these signaling pathways comprehensively. Further studies about domains in *AtCNGCs*' structure and their interaction with the predicted ligands require a clear understanding. The effect of these interactions on the functionality of these channels can significantly contribute to plant CNGCs research. The outcomes of these studies can benefit the generation of pathogen-resistant and stress-resistant crops.

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