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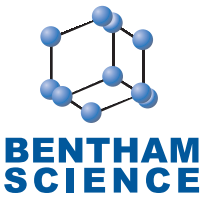

BENTHAM SCIENCE

Role of Cyclic Nucleotide Gated Channels in Stress Management in Plants

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Abstract: Tolerance of plants to a number of biotic and abiotic stresses such as pathogen and herbivore attack, drought, salinity, cold and nutritional limitations is ensued by complex multimodule signaling pathways. The outcome of this complex signaling pathways results in adaptive responses by restoring the cellular homeostasis and thus promoting survival. Functions of many plant cation transporter and channel protein families such as glutamate receptor homologs (GLRs), cyclic nucleotide-gated ion channel (CNGC) have been implicated in providing biotic and abiotic stress tolerance. Ion homeostasis regulated by several transporters and channels is one of the crucial parameters for the optimal growth, development and survival of all living organisms. The CNGC family members are known to be involved in the uptake of cations such as Na^+, K^+ and Ca^{2+} and regulate plant growth and development. Detail functional genomics approaches have given an emerging picture of CNGCs wherein these protein are believed to play crucial role in pathways related to cellular ion homeostasis, development and as a 'guard' in defense against biotic and abiotic challenges. Here, we discuss the current knowledge of role of CNGCs in mediating stress management and how they aid plants in survival under adverse conditions.

Keywords: Abiotic stress, Biotic stress, Cations, Calcium signaling, Channels, Stress tolerance.

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1. INTRODUCTION

One of the critical questions that need to be answered before it is too late is whether the rate of increment in crop yield would be substantial to feed the world's ever increasing population, which is estimated to cross nine billion by 2050 [1]. According to projections from the Food and Agriculture Organization (FAO), in order to feed the ballooning population the average world cereal production must reach from its present 3 ton per hectare to 5 ton per hectare. Expanding population together with unprecedented environmental changes has caused food insecurity for the mankind [2]. To ensure food for every mouth is one of the biggest challenges we are facing today that too when the changing environmental conditions have led to food production threat. In general, both biotic and abiotic stresses are the major factors that limit crop yields. The adverse effects of abiotic stresses such as drought, cold, high temperature, salinity, osmotic stress and nutrient deficiency are affecting the overall crop production [3, 4]. Moreover, soil salinity is one of the major abiotic stresses, which has negative impact on plant productivity worldwide. Many of the crop species whose products are consumed by countless people for survival are negatively affected by high salinity [5, 6].

Reports highlight that around 80 million hectare (ha) of cultivated lands are affected by soil salinity [7]. Therefore, the important challenges for agricultural production in ever-shrinking area of arable land are to increase water-use efficiency (WUE) and salt tolerance in economically important plant species. Adaptation to such adverse environmental conditions is crucial not only for the survival of living beings but also eventually for entire biosphere. For the same, understanding the mechanisms that how plants respond to the adverse climatic conditions and adapt to the changing environments will enable us to develop plants that can cope well in non-conducive growth and development conditions. Adaptation and tolerance of plants to adverse conditions need fine-tuned cumulative effects of a number of biochemical and physiological changes, which in turn depend on the gene expression under those conditions [8]. In the years to come, changes in climatic variability will lead to more frequent extreme conditions [2] which in turn will require directed adaptations of crop species on an unprecedented scale in order to sustain agricultural production. Drought, salinity, high and low temperature extremes are among the major stresses that limit plant growth and productivity [9, 10]. In general, extreme low temperature causes mechanical constraints, whereas salinity and drought result in disruption of ionic and osmotic equilibria of the cell. Myriad of stress conditions lead to activation of both mechanisms of acclimation and adaptation by the plant. Regulation of stress-related genes forms the basis of molecular control mechanisms that mediate abiotic stress tolerance. Plants, being sessile are endowed with 'sense organs' that can perceive environmental changes and adequately respond to stress. Since the nature of stresses is complex; multiple sensors, rather than a single sensor, are deployed for perception of stress stimuli. Recognition of a stress stimulus leads to the onset of a signal transduction

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cascade that results into generation of secondary messengers such as calcium (Ca^{2+}) [11, 12]. These secondary messengers relay the signal and ultimately modulate the expression of stress-related genes. Extensive research during the past decade has established that various stresses result into Ca^{2+} accumulation in the cytosol, which play an important role as a secondary messenger in modulating a multitude of physiological processes and gene expression that ultimately leads to stress adaptation [12]. Various kinds of Ca^{2+} channels are known in plants that contribute to the Ca^{2+} accumulation in the cytosol and cyclic nucleotide-gated channels (CNGCs) are one of them. The CNGCs are ligand-gated cation channels localized mostly in the plasma membrane [13, 14]. Plant CNGCs consist of cytoplasmic C-terminal calmodulin binding domain (CaMBD) and cyclic nucleotide-binding domain (CNB) whereas N-terminal serves as hexa-transmembrane (TM) domain. The TM domain forms a passage to facilitate cation transport [15]. The plant CNGC genes were for the first time identified in barley [16] and later on search was extended to several other plant species including *Arabidopsis thaliana* [17, 18] and tobacco [19]. Genome-wide identification of CNGC gene family was also carried out in *Arabidopsis* [20], rice [21], *Populus trichocarpa* [22], pear [23], moss (*Physcomitrella patens*) and some algae [24, 25].

The *Arabidopsis CNGC* gene family consists of 20 members that are divided into four groups namely-I, II, III, IVa and IVb as per their phylogenetic relationship [20]. Recently, Saand *et al.* (2015) have identified CNGC family in the economically important crop plant tomato [26]. Through bioinformatics and experimental analyses they identified 18 tomato CNGCs [26]. However, it is quite surprising that in tomato CNGC members are less than *Arabidopsis,* which contains 20 members [20] albeit the genome size of tomato is much larger than *Arabidopsis*. According to Saand *et al.* (2015) possible reasons could be that retrotransposons and retroviruses disrupt *CNGC* genes and lead to loss of full-length *CNGC* members in tomato genome [26]. Moreover, the lesser number of *CNGC* genes in tomato could be due to relatively more conserved genomes among the members of *Solanaceae* family, including tomato [27]. Additionally, the genome size of rice and pear is substantially larger than that of *Arabidopsis* but they consist of only 16 and 21 *CNGC* genes respectively [21, 23]. The genome size of rice is nearly three times as that of *Arabidopsis* [28] but contains only 16 CNGC genes. The reason could lie in the variable status of whole genome duplications in *Arabidopsis* and rice [29, 30].

Studies on animal models have indicated that plant CNGCs might facilitate Ca^{2+} influx. Reports are also suggestive of their activation by direct binding of cyclic nucleotides (CNs) such as cAMP and cGMP to the CNB domains and inhibition by binding of calmodulin (CaM) to the calmodulin binding (CaMB) domain [13, 31]. Upon activation they promote influx of the Ca^{2+} into the cytosol [32, 33]. Recent report from an electrophysiological study provides evidence that *AtCNGC18* functions as a Ca^{2+}-permeable divalent cation-selective channel and its activity is modulated by cGMP and cAMP in HEK293T cells [34]. Further insight into the regulation of *CNGCs* was provided by Zhou *et al.* (2014) where they reported that *AtCNGC18* is activated by calcium-dependent protein kinase, AtCPK32 in *Xenopus laevis* oocytes [35].

The plant CNGCs are reported to mediate numerous biological processes ranging from plant development and stress tolerance [32] to disease resistance [36, 37] (Table **1**). CNGCs are believed to be regulators of myriad of plant development and stress tolerance pathways. For instance, *AtCNGC1* is involved in Ca^{2+} uptake [38]; *P. patens CNGC (PpCNGCb)* and its *Arabidopsis* ortholog (*AtCNGC2*) play essential role in thermotolerance [39]; *AtCNGC3* helps in seed germination [40]; *AtCNGC6* contributes in Ca^{2+} uptake when plants are challenged with heat stress and thus mediates acquisition of thermotolerance [41]; *AtCNGC10* is involved in plant growth [42]; *AtCNGC16* and *AtCNGC18* are crucial for pollen fertility under stress and pollen tip growth respectively [43, 44] whereas *AtCNGC19* and *AtCNGC20* are associated with salt tolerance [45].

Genetic dissection has divulged the important role of four *Arabidopsis CNGC* genes, *AtCNGC2*, *AtCNGC4*, *AtCNGC11* and *AtCNGC12* in plant disease resistance. The *AtCNGC2* mutant, *defense no death1* (*dnd1*) displays reduced hypersensitive response (HR) development but at the same time exhibits enhanced basal resistance to *Pectobacterium carotovorum* [46]. Simultaneously, the *dnd1* mutant shows *R* gene-conferred resistance to avirulent pathogens with accumulation of salicylic acid (SA) [47, 48]. Studies conducted by Ali *et al.* (2007), unveiled that *AtCNGC2* promotes Ca^{2+} current to produce nitric oxide (NO), which causes HR development in response to pathogens [33, 49]. Moreover, mutation in *NEC1,* which is a homolog of *AtCNGC4* in barley, causes similar symptoms to *dnd2/hlm1* [50, 51]. As far as plant defense signaling against pathogens is concerned, *AtCNGC2* and *AtCNGC4* seem to be regulating the same pathway [52]. Thus, the above-described evidence unambiguously supports that CNGCs are essential in plant defense.

Passive movement of ions is facilitated by ion channel that depends on membrane electrochemical gradient for that ion [33]. Earlier reports have determined the amount of Ca^{2+} in the cell wall and vacuole in 1-10mM range while in the cytosol it is maintained at much lower level ranging 100-200nM. This variation is tightly controlled and maintained well below 1μM even during signaling events [53, 54]. This difference in the electrochemical gradient between the cytosol and the two major Ca^{2+} pools of the cell favors passive movement of Ca^{2+} into the cytosol through the gated channels like CNGCs. CNGCs are intrinsic membrane protein ion channels that facilitate ion diffusion across the membranes [33]. Additionally, these channels are gated and permeation of ions through their pore is conditional *i.e.*, ion diffusion can take place only when the factors that regulate the conductance lead to physical changes in the protein structure so as to facilitate the ion movement through them [33].

ANIMAL CNGCs: INTRODUCTION

In animals, CNG channels play important role in both visual [55] and olfactory [56] signal transduction. These are nonselective cation channels and were first identified in retinal photoreceptors [57] and olfactory sensory neurons (OSNs) [58-60]. But growing body of evidence suggests that CNG channels are not unique to photoreceptors and OSNs, they are expressed in other neurons and non-neuronal tissues

Table 1. ***Arabidopsis*** **CNGCs and their function in various physiological processes.**

Gene	Function	Reference
AtCNGC1	Lead (Pb^{2+}) tolerance	[149]
AtCNGC2	Involved in pathogen defense, NO (nitric oxide) generation, floral transition and thermotolerance	[48, 52, 159, 39]
AtCNGC3	Involved in germination and cation transport	[40]
AtCNGC4	Imparts enhanced broad-spectrum resistance against virulent pathogens, involved in constitutive expression of PR genes and floral transition	[79, 52]
AtCNGC5	Ca^{2+} conduction in the guard cells	[31]
AtCNGC6	Mediates heat-induced Ca^{2+} entry and induces expression of heat shock proteins (HSPs) and development of acquired thermotolerance	[31, 41]
AtCNGC7	Important for male reproductive fertility	[44]
AtCNGC8	-do-	[44]
AtCNGC9	Not defined (ND)	
AtCNGC10	Negative regulator of salt regulation, mediates Ca^{2+} and Mg^{2+} ion conduction, involved in numerous growth responses and starch accumulation	[112, 42]
AtCNGC11	Positively regulates salicylic acid (SA)-dependent/R gene- mediated pathogen defense responses	[157]
AtCNGC12	Involved in the development of resistance against avirulent fungal pathogen, suppression of *cngc11/12* (*cpr22*) phenotype	[157]
AtCNGC13	ND	
AtCNGC14	ND	
AtCNGC15	ND	
AtCNGC16	Crucial for stress tolerance in pollen reproductive development	[44]
AtCNGC17	Involved in growth regulation in association with phytosulfikine, H^{+}-ATPase and BAK1	[160]
AtCNGC18	Critical for polarized tip growth of pollen	[43]
AtCNGC19	Involved in salt stress	[45]
AtCNGC20	-do-	[45]

as well [61]. These ion channels are directly activated by binding of cyclic nucleotides such as cAMP and cGMP. CNG channels are member of a heterogeneous gene superfamily ion channels that exhibit a common transmembrane topology and pore structure [57]. It harbors a COOH-terminal region, a binding domain for nucleoside 3', 5'-cyclic monophosphates (cNMPs) [61]. Similar to K^{+} channels, CNG channels are composed of heterotetrameric complexes consisting of two or three different types of subunits. In animals, there are three different channels in rod and cone photoreceptors and in OSNs. These are encoded by six different genes-coding for four A subunits (A1 to A4) and two B subunits (B1 and B3). Salient features of these channels, such as ligand sensitivity and selectivity, ion permeation and gating are defined by the subunit composition of the respective channel complex. The roles of CNG channels have been unequivocally established in retinal photoreceptors and in OSNs. Furthermore, the function of invertebrate homologs found in *Caenorhabditis elegans*, *Drosophila*, and *Limulus* is not well explored, except for two subunits of *C. elegans* that play a role in chemosensation [61].

Being nonselective cation channels, CNGCs do not discriminate well between alkali ions and even allow divalent cations particularly Ca^{2+} to pass through [61, 62]. Entry of Ca^{2+} through CNG channels is required for both excitation and adaptation of sensory cells. Besides other factors, the channel activity is modulated by Ca^{2+}/calmodulin complex and by phosphorylation. Consequences of CNG channel gene mutations are retinal degeneration and color blindness [61]. Notably, mutations in the A and B subunits of CNG channel expressed in human cones can result in various forms of achromatopsia [61].

ANIMAL CNGCs: FUNCTION

Cyclic nucleotides (CNs) directly modulate the CNG channel activity by binding to a site on the channel protein. The activation of the channel requires a steep ligand concentration, suggesting that several, most probably four, molecules of the ligand are needed to fully open the channels [63]. Generally, all CNG channels respond to some extent to both cAMP and cGMP [61]. However, there is also some

degree of ligand selectivity. CNG channels present in rods and cones can discriminate between cAMP and cGMP, whereas channels in chemosensitive cilia of OSNs more or less respond equally to both ligands [61]. Since CNG channels poorly discriminate between alkali ions they conduct mixed inward currents carried by Na^+ and Ca^{2+} [63]. For conduction, the ions bind to a site inside the channel pore. The residing time at this binding site is significantly more for Ca^{2+} than monovalent cations. Consequently, Ca^{2+} hinders the current of the more permeable Na^+ [63]. The higher Ca^{2+} permeability and the concomitant blockage of Na^+ conduction by divalent cations is crucial for channels' functioning, for example, the ability of rod photoreceptors to detect single photons and to adapt to steady illumination [64-66].

The physiological functions of CNG channels have been established not only in rod and cone photoreceptors but also in extraretinal photoreceptors and in sensory neurons of the olfactory epithelium [61]. Invertebrate and vertebrate ciliary photoreceptors, whether depolarizing or hyperpolarizing, use cGMP-signaling pathways. The potential targets of these pathways are CNG channels that either open or close in response to light [61]. Chemosensory cells found in vertebrates can be divided into three groups: olfactory sensory neurons, neurons of the vomeronasal organ and taste receptor cells [61]. The involvement of CNG channels in signal transduction has been proposed for each of these different senses. Studies in the nematode *C. elegans* suggest that CNG channels might play important role in chemotaxis to odorants and in salt in this model organism [57, 61]. In addition to these, cyclic nucleotides are considered to be key elements of cellular signaling in sperm of both vertebrates and invertebrates. cAMP and cGMP via CNG channels mediate many cellular responses, including acrosomal exocytosis, swimming behavior and chemoattraction [61, 67]. The swimming activity of sperm is controlled by factors, commonly short peptides that are released by the egg or cellular structures of the oviduct [61]. In some of the species, chiefly, marine invertebrates wherein external fertilization is common, the amino acid sequence of the peptides and their counterpart membrane receptors present on the surface of the sperm have been identified [64-66]. For instance, a short peptide called speract released from the sea urchin *Strongylocentrotus purpuratus*, is involved in the activation of a receptor-type GC and in turn stimulates an increase in the intracellular cGMP concentration [61, 64]. Moreover, speract can also give rise to an increase of $[Ca^{2+}]_i$. This evidence indicates that cGMP activates Ca^{2+} entry into sperm cell. Because of their high Ca^{2+} permeability, CNG channels are among the favorite candidates for the Ca^{2+} entry pathway.

PLANT CNGCs: A CATION/Ca^{2+}-CONDUCTING CHANNEL

Unlike animals, higher plants do not possess canonical genes encoding voltage-gated Ca^{2+} channels [22, 24, 37]. The *Arabidopsis* genome contains 57 genes encoding cation-conducting channels, out of which 20 members belong to cyclic nucleotide-gated channel (CNGC) family [32, 33]. *CNGC* genes have been identified in several plant species including barley [16], *Arabidopsis* [17, 18] and tobacco [19].

CNGCs have been reported to conduct cations and are activated by binding of cyclic nucleotide to the cytosolic half of the channel. Previous reports have revealed that CNGCs facilitate inward Ca^{2+}-conduction into the cell that occurs during Ca^{2+} signal transduction in plants [24, 37, 68-70]. Functional analysis in heterologous expression system yeast [43, 71, 72] and phenotypic analysis involving translational arrest of their expression in plants [14, 43, 72-78] substantiate the notion that some of the CNGCs form Ca^{2+}-conducting channels. These include CNGC 1, 2, 10, 11, 12 and 18. Subsequently, CNGC 5 was also found to be mediating Ca^{2+}-conduction [38].

As already mentioned, structurally plant CNGC is similar to Shaker channels. It has also been known that contrary to animal CNGC, plant CNGC has overlapping binding domains of CN and CaM [17-19], which may facilitate crosstalk between CaM and CN signaling [19]. Ligand in the form of cGMP or cAMP is required for their gating. In *Arabidopsis*, AtCNGC1 and AtCNGC4 (HLM1) have been proved to have equal permeability for Na^+ and K^+ [75, 79, 78]. Noticeably AtCNGC2, which is unique in having Aln-Asn-Asp selectivity filter, is highly selective for K^+ over Na^+ [80, 75]. Due to its preferential selectivity for K^+ and higher expression in roots [81], it is speculated that AtCNGC2 may be directly involved in K^+ uptake.

In the quest of unraveling selectivity of plant CNGCs for different cations, its expression and characterization in heterologous system was attempted in *Saccharomyces cerevisiae* mutants deficient in cation uptake and by electrophysiological means. Yeast mutants devoid of both of their K^+-uptake transporters, *trk1*, *trk2* and a salt-sensitive yeast strain with disruption in the major Na^+ extruding pumps ENA1-4 were used to test complementation of K^+ and Na^+ uptake respectively by the channels. In the studies, the *Arabidopsis* isoforms AtCNGC1 and AtCNGC2 could partially complement the *trk1*, *trk2* yeast mutant even in the absence of permeable cNMPs [38]. Contrary to the latter report, Leng *et al.* (1999) showed that AtCNGC2 could partially complement the yeast mutant at low K^+ concentration only in the presence of membrane-permeable cAMP [71]. Leng *et al.* (1999) reported the foremost functional characterization of a plant CNGC using electrophysiological technique.

Additionally, AtCNGC3 could also partially complement a yeast mutant lacking the K^+ transporters [40]. Similar to K^+ uptake characterization, a salt-sensitive yeast strain with disrupted major Na^+ extruding pumps ENA1-4 was used by Gobert and collegues [40] to test the Na^+ permeable function of AtCNGC3. The yeast mutant expressing AtCNGC3 was more sensitive to high salt and accumulated more Na^+ than the cells with the empty control vector, implying that AtCNGC3 forms a functional Na^+ permeable channel in these yeast cells [40].

Kurosaki *et al.* (1994) could show that the activation of a plasma membrane calmodulin contributed to the termination of cAMP-dependent Ca^{2+}entry in cultured carrot cells [74]. Application of heterologous expression systems such as HEK293 cells or *Xenopus* oocytes together with electrophysiological studies has helped in unraveling the functional properties of CNGCs [71]. Complementation assays with

plant CNGCs in various bacterial and yeast mutants compromised in K^+, Ca^{2+} or Na^+ uptake have also provided with clues regarding the ion selectivity and permeability of these channels. The structural modeling of plant CNGCs on the basis of bacterial K^+ selective channels defines a triplet of amino acids in the P-loop region as selectivity filter. Most of the *Arabidopsis* CNGCs possess GQN triplet (AtCNGC1, 3, 10-15, 17 and 18) while few members also harbor GQG triplet (AtCNGC5-9) [75]. Contrary to these, AtCNGC19 and AtCNGC20 harbor AGN, AtCNGC16 has GQS, AtCNGC2 has AND, and AtCNGC4 possesses the GN-L motif [75]. The CNGCs permeable for Ca^{2+} - AtCNGC1, 2, 10, 11, 12 and 18 are provided with GQN triplet.

PLANT CNGCs: REGULATION BIOLOGY

The cyclic nucleotides cAMP and cGMP are ubiquitous molecules that play important roles in regulating an array of cellular processes including gene expression and signal transduction [76, 77]. The cyclic nucleotides can bind to two functional domains in the proteins, the GAF (cyclic GMP, adenylyl cyclase, Fh1A) and the cyclic nucleotide binding (CNB) domain [78]. The CNB domain is chiefly present in two groups of the cation permeable channel family in plants, the cyclic nucleotide gated ion channels (CNGCs) and the shaker-type potassium channels [22, 78].

In animals, CNGCs possess a CaM-binding domain at their N-terminal, which interacts with the CNBD in the C-terminal portion of the protein, resulting into the channel inactivation [32, 82]. However, this is not true for plants wherein, the CaM-binding domain (CaMBD) overlaps with one of the three conserved helices of the CNBD [32, 83]. This suggests that even though functionally both plant and animal CNGCs share similar response towards Ca^{2+}/CaM albeit their interaction mechanisms with this complex differ considerably [32]. For instance, in the presence of Ca^{2+}, CaM binds to the plant CNGC at the αC helix of the CNBD and thus perturbs channel gating by cyclic nucleotide monophosphates (cNMPs) [32, 83].

AtCNGC10 is yet another example where plant CNGC activity modulation involving cNMPs and CaM in a heterologous system was exemplified [32, 84]. The functional aspect of CaM binding domain of CNGCs has been shown in the tobacco NtCBP4 [83], the barley HvCBT1 [16], and the *Arabidopsis* channels AtCNGC1 [17], AtCNGC2 [17, 18, 75] and AtCNGC10 [84]. The effects of the interaction between Ca^{2+}/CaM and the CaM binding domain have been well demonstrated. The CaM binding to AtCNGC2 controls a Ca^{2+}-dependent feedback regulation by decreasing the affinity of the cyclic nucleotides for the CNB domain [75]. The expression of a truncated version of AtCNGC1 that lacks a portion of CaM binding domain resulted in an increase in the intracellular K^+ concentration in the *trk1/trk2* yeast mutant deficient in K^+ uptake [85]. Recently, Zhou *et al.* (2014) has reported that CNGC may undergo post-translational modification such as phosphorylation [35]. The evidence indicates that a calcium-dependent protein kinase, CPK32, controls pollen tube growth. Overexpression of CPK32 disrupted the polar pollen tube growth and there was excessive accumulation of Ca^{2+} at the tip of the pollen tube. CNGC18 could interact with CPK32 and co-expression of CPK32 and CNGC18 in *Xenopus* oocytes resulted in the activation of CNGC18. Overexpression of CPK32 displayed the same phenotype as that of CNGC18 overexpression. Furthermore, co-expression of CNGC18 and CPK32 had a synergistic effect resulting more severe depolarization of pollen tube growth. Thus, it appears that there is a feed-forward mechanism wherein a calcium-activated CPK32 activates CNGC18, which further promote Ca^{2+} entry during polar growth of pollen tubes [35].

COMPARATIVE ACCOUNT OF PLANT AND ANIMAL CNGCs

CNGCs exhibit varying degree of ion selectivity for conduction. Their role has been implicated in numerous signaling pathways and allows diffusion of divalent and monovalent cations including Ca^{2+} and K^+. Presence of CNGCs has been known in both plant and animal cells (Table **2**) with variable numbers and they are typically localized to the plasma membrane. Some recent studies have also documented their presence in prokaryotes [15].

Table 2. Variable number of CNGCs present in plant and non-plant system.

Species Name	Number of CNGCs Present	References
Homo sapiens	**6**	[24]
Arabidopsis thaliana	**20**	[17]
Oryza sativa	**16**	[21]
Solanum lycopersicum	**18**	[26]
Physcomitrella patens	**8**	[24]
Chlamydomonas reinhardtii	**3**	[24]
Micromonas RCC299	**2**	[24]
Thalassiosira pseudonana	**2**	[24]
P.trichocarpa	**12**	[22]
Pyrus bretchneideri Rehd.	**21**	[23]

All eukaryotic CNGC polypeptides possess CNB domain and CNBD as well as six transmembrane/one pore tertiary structures [15]. The pore and CNBD sequences of plant cyclic- nucleotide gated channels not only differ from the pore and CNBDs within the plant kingdom but also from the pore and CNBD regions of animal CNGCs [15]. Bioinformatic analyses have revealed that the most conserved region of the CNGC CNBD is a phosphate-binding cassette (PBC), which binds the sugar and phosphate moieties of the cNMP ligand [86]. Adjacent to the PBC is also a conserved region called as "hinge" and is thought to contribute to ligand efficacy and selectivity [87]. Activity of animal CNGCs is regulated allosterically by CaM and cyclic nucleotides (cAMP and/or cGMP). In the presence of cytosolic Ca^{2+}, CaM binds to CaMBD of animal CNGCs at the N-terminus thereby closing the CNG channel and preventing ion conduction. Cyclic nucleotides can bind the C-terminus of both plant and animal

CNGCs. cNMP binding to the CNBD of CNGC polypeptides activates the channel, opening it and thereby enabling cation conductance. The sequences of amino acids that line the pore selectivity filter of P-loop channels define the relative conductance for Na^+, K^+ and Ca^{2+}. An important structural aspect of plant CNGCs is their CNBD. From evolutionary point of view, the presence of CNGC coding sequences with CNBD in animals and plants is intriguing. Reports suggest that unicellular fungi lack channels with such CNBDs [88, 89]. While CNBD of plant CNGCs overlaps with a CaMBD at the C-terminus, and CaM in the presence of cytosolic Ca^{2+} prevents cNMP activation of plant CNGCs [71], there is no corresponding CaMBD in bacterial CNGC sequences [86]. In case of animals the functional CaMBD is located to the distal end of the CNBD, near the N-terminus [90]. As mentioned earlier, CaM binds to and regulates the conductance of animal CNGCs and this is also true for plant CNGCs. However, in plant CNGCs, the amino acids that form the CaM binding domain overlaps with the region of the polypeptide that forms the CNBD. So far, no CNGC-specific motifs have been reported, however, Jackson *et al.* (2007) aligned different animal CNGCs, particularly their PBC and hinge regions from which the motif FGE-[IT]-[CIA]-LL-X(3,4)-[RK]-R-X-A-SV-X(11)-[SH]-[VRA]-[HNQ]-X-[LV]-[LA] (the animal CNGC hinge sequence spans from the conserved serine (S) to the C-terminus of the motif) was identified [91]. Zelman *et al.* (2012) noted that such a motif is not present in plants and postulated that some of the functionally critical residues might be conserved [15]. The plant CNGC hinge occurs in between the CNBD and CaMBD while in animals the hinge region occurs within the CNBD itself. Moreover, plant CNGC hinge region lacks the conserved proline (P) that was crucial for gating in animal CNGCs [91].

FUNCTIONAL ROLE OF PLANT CNGCs

For CNGCs, substrate specificity has far-reaching implications for instance in osmotic adjustment and Ca^{2+} signaling. CNGCs are known to play multifaceted roles in plants ranging from ion homeostasis, nutrient uptake, heavy metal tolerance, floral transition, growth and development to providing plant immunity (Table **1**). Here, in the following subsections we try to find the possible roles of CNGCs in stress management that includes their implications in abiotic and biotic stress management.

1. Role of CNGCs in Salt Tolerance

Salinity is one of the major abiotic stresses affecting a significant total arable land area globally and results in billion dollar losses in food crop production all across the world [92]. When cellular Ca^{2+} is scarce, plants are more prone to damage by low pH or high saline conditions [93]. A number of reports from previous studies suggest that external and apoplastic Ca^{2+} directly mitigates symptoms manifested by ionic stresses or mineral toxicities like H^+, Na^+, Al^{3+} and Cl^-. Not only that, Ca^{2+} also establish an optimal and favorable K^+: Na^+ ratio under salinity stress [93, 94]. It has been proven that intracellular Ca^{2+} signal transduction via a calcineurin-like module mediates the beneficial adaptive effect of Ca^{2+} on plants towards salt tolerance [95-98]. High saline conditions invoke Ca^{2+}-dependent signaling pathway, which has been deciphered and established in detail to mediate cellular Na^+ homeostasis and salt tolerance, indicating unambiguously that Ca^{2+} conducting membrane proteins are closely related to plant salt tolerance (Fig. **1**). It has also been proven that CaM activation is necessary in Ca^{2+}-induced proline accumulation in calli and supplementation of Ca^{2+} to media mitigates the callus growth inhibition under salt stress [99]. Thus, the above facts demonstrate that CaM might be working hand-in-hand with Ca^{2+} and involved in Ca^{2+}-signals decoding elements in plant responses to salt stress [100]. Studies done with barley roots have demonstrated the activation of tonoplast localized H^+-ATPase and the modulation of Na^+ and K^+ acquisition under salt stress may be related to Ca^{2+}-CaM system, strengthening the assumption that calcium signal decoding elements participate in plants responses towards salt stress tolerance through cytosolic [Ca^{2+}] regulation [101]. Many reports from Zhu and co-workers and others highlighted that in *Arabidopsis*, network of calcium signal transduction is linked with the activation of the salt overly sensitive (SOS) signaling network which helps in regulating Na^+ and K^+ homeostasis [91, 95, 96, 100-102].

Several decisive experiments gave substantial evidence that some physiological functions and molecular features of the nonselective cation channels (NSCCs) such as CNGCs are directly related with a multitude of stress adaptive responses, growth and development, acquisition of nutrients and Ca^{2+} signaling. Hence, in conclusion, many types of Ca^{2+} permeable elements including CNGCs, Ca^{2+}-ATPases and CAXs are involved in plant responses to salt stress via regulating cytosolic [Ca^{2+}] and thus modulate cellular and intercellular Ca^{2+} signal cascade and improve tolerance [88, 89, 103-106].

As stated above, non-selective cation channels (NSCCs) are ubiquitous in both plasma and endomembranes particularly at the tonoplast of plant cells. There are about 40 putative NSCCs in *Arabidopsis* genome [20, 82, 107]. NSCCs are known to show a preferential conduction of cations over anions but they usually do not discriminate strongly between cations [92, 107-110]. Physiologically, NSCCs function in low-affinity uptake. Activity of NSCC might be regulated by a large number of factors that include depolarization-activation, hyperpolarization-activation, voltage variation, Ca^{2+} activation, mechanical perturbance, binding of cyclic nucleotides and glutamates [92, 110]. It is well established that transcript level of a number of NSCC family members are modified by salinity [92, 111] and it is assumed that this may impart benefits during salt stress as it will help in decreasing the osmotic potential of cell, thereby alleviating water stress. If the excess Na^+ is not sequestered into the vacuole, the result can be devastating because of severe Na^+ toxicity in the cytosol. CNGCs are activated either by cAMP, cGMP, Ca^{2+} or CaM [20, 92, 108, 80], resulting in conduction of cations into the cell [79]. Being categorized as non-selective, they allow influx of K^+, Na^+ and Ca^{2+} equally into the cell [92, 79]. CNGCs exhibit ubiquitous expression in all tissues [80] and are localized to the plasma membrane [19, 92]. There is a great functional diversity among 20 different members of CNGC in *Arabidopsis* ranging from cell signaling [107] to cation uptake by roots [111]. Moreover, for several members of CNGC family, data related to their physiological functions are available [79, 80], which reveal

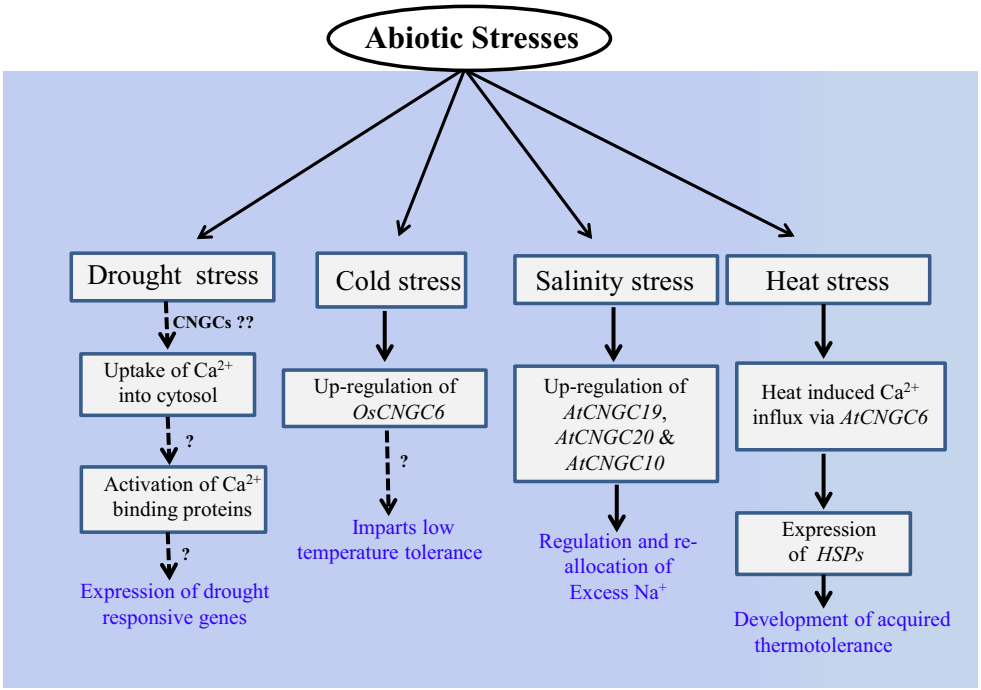

Fig. (1). A schematic representation of CNGC-mediated signaling cascade generated in response to abiotic stress for tolerance mechanism. Up-regulation of CNGCs and Ca^{2+} influx mediated by them have been validated in abiotic stresses such as heat, cold, salinity and (possibly) drought (?). The downstream molecules in the form of Ca^{2+}sensor/relay together with other components impart tolerance under abiotic stress conditions. (Broken arrows indicate possible pathway while bold arrows represent pathways demonstrated by experimental proofs).

that almost all characterized CNGCs have potential to transport K^+ and Na^+. Analysis of transcriptome data has revealed that the transcript levels for a few CNGCs like *CNGC1*, *CNGC19* and *CNGC20* increase under salinity in *Arabidopsis* roots, while transcript levels of *CNGC19*, *CNGC3* and *CNGC8* increased in shoots [82, 104]. Kugler *et al.* (2009) through GUS, GFP and luciferase reporter assays monitored the expression of *CNGC19* and *CNGC20* genes from *Arabidopsis* in response to developmental cues and salt stress [45]. They found that *CNGC19* and *CNGC20* were differentially expressed in roots and shoots. The *CNGC19* gene was predominantly expressed in roots at early growth stage while *CNGC20* showed highest promoter activity in mesophyll cells surrounding the veins. The expression of *CNGC20* gradually increases during development and reach to saturation in mature and senescing leaves. Both genes showed upregulation in the shoot in response to elevated NaCl concentration. During the elevated salt conditions, expression of *CNGC19* was entirely absent in the root whereas it expresses strongly in the shoot with specific upregulation observed during the time frame of 6-72 hours. Similar to *CNGC19*, salt dependent induction of *CNGC20* was also observed in the shoot [45]. In summary, these results strongly indicate that both channels are involved in the salinity response of different cell types in the shoot. Since both genes are upregulated within hours, they could assist the plants to cope with toxic effects caused by salt stress probably by mediating the re-allocation of excess sodium to other parts of the plants [45]. Beside *CNGC19* and *CNGC20*, recently role of *CNGC10* has also been identified in the response to salt stress. In the study conducted with *cngc10* T-DNA insertion mutant, the mutant displayed better tolerance to salt than wild type during seed germination and seedling growth by regulating Na^+ transport [112].

2. Role of CNGCs in Drought Tolerance

Drought is a major stress factor that inhibits plant growth, development and overall productivity. Several studies have shown that exogenous application of Ca^{2+} has the promising potential to enhance plant drought tolerance, prevent the synthesis of activating oxides, protect the plasma membrane architecture, promote optimal photosynthesis and modulate the metabolism of phytohormone and other important chemicals. Moreover, being secondary messenger, cellular Ca^{2+} also transmits drought signals, thus modulating the physiological responses invoked by drought stress factor [77, 105, 106, 113-117]. It has been known that Ca^{2+} treatment increases stability of cellular membranes and provide protection against lipid peroxidation in drought stress samples and thus result in the increase in drought tolerance of rice seedlings. In wheat, Ca^{2+} appeared to mitigate the damaging effect of stress by increasing osmoprotectants like proline and glycine betain content, thus improving the water status and growth of seedlings and reducing the injury to membranes [54, 100, 118-121]. The above-mentioned results are indicative that Ca^{2+} plays important roles in plant responses to drought stress (Fig. **1**).

Now-a-days, water-use efficiency (WUE) is being given much more attention as a desired agronomic trait regarding plant drought tolerance and yield [103, 122-124]. In plant breeding programmes, in order to develop plants tolerant to drought stress, biomass productivity and WUE are considered as fundamental agronomic characteristics [125,

126]. As a well-established signaling molecule, Ca^{2+} is undoubtedly closely related to various abiotic stress tolerance mechanisms in plants. In the signaling networks, some specialized proteins called Ca^{2+} signal decoding elements mainly regulate the cellular Ca^{2+} signal transduction pathways. It has also been proved that regulation of stomatal aperture is Ca^{2+} dependent with stimuli such as ABA and influx of extracellular Ca^{2+} under certain conditions causing a rise in Ca^{2+}. This upsurge of Ca^{2+} precedes the loss of turgor by the surrounding guard cells. It has also been reported that ABA induces an increase in Ca^{2+} in guard cells, which precedes the stomatal closure. Thus, it is believed that Ca^{2+} causes reduction in stomatal aperture [127-130]. ABA triggers an increase in cytosolic Ca^{2+} in guard cells and this include Ca^{2+} influx across the plasma membrane through Ca^{2+} channels and release from intracellular Ca^{2+} stores [131-133]. An array of studies has shown that cytosolic-free concentration of Ca^{2+} plays a central role in stomatal movement and the change in Ca^{2+} mediates stomatal opening or closing [103, 134-136]. Furthermore, studies have established a close relationship between cytosolic Ca^{2+} oscillation and stomatal aperture status [130, 137]. Ca^{2+} dissolved in water in the apoplast is transported mainly through the transpiration stream from root to the shoot part and rate of transpiration is governed by the stomatal conductance. These results show a close association of Ca^{2+}, rate of transpiration and stomatal conductance. Therefore, from studies of Ca^{2+} channel proteins in Arabidopsis and other plants, we can conclude that the genes encoding these proteins play important role in plant responses to various abiotic stresses conferring toleranc to plants [138-140]. So far only few studies have predicted the role of CNGCs in drought stress regulation. The genetic study of *AtCNGC16* provided evidence that this gene is critical for pollen fertility under the conditions of heat stress and drought. Two transfer DNA (T-DNA) insertion mutants of *cngc16* showed greater than 10-fold stress-dependent reduction in pollen fitness and seed set. This phenotype was fully rescued by expressing *CNGC16* transgene in the pollen [44]. Hence, it can be speculated that somehow CNGC may be involved in mediating drought stress tolerance.

3. Role of CNGCs in Cold Tolerance

It is generally believed that Ca^{2+} channels play substantial role in the root hair growth and in acclimatization of chilling-tolerant plants to low temperature [100]. It has been reported that features like activity and stability of Ca^{2+}-ATPases under 2ºC low temperature are the key determinants in the development of cold resistance in winter wheat [129, 141-143]. In plant species such as alfalfa and Arabidopsis, it has been demonstrated that Ca^{2+} entry into the cytoplasm serves as a signal transduction component for the activation of genes involved in imparting low temperature tolerance [144]. Several researchers have proved that addition of Ca^{2+} specific chelators inhibits cold acclimatization and expression of cold responsive genes [120, 136]. Studies on some of the tonoplastic Ca^{2+}/H^{+} exchanger (AtCAX1) mutants having reduced activity suggests that CAX1 plays role in the development of cold acclimation response [54]. Some reports reveal the fact that there is transient increase in Ca^{2+} concentration in cold-insensitive plants when exposed to cold conditions, suggestive of the notion that Ca^{2+} acts as a second messenger during cold acclimation. Not only that, Bartels and Sunkar (2005) [145], reported that distribution and accumulation of Ca^{2+} is different among the various tissues and organs in *Chorispora bungeana*, which could play an important role in its cold-hardiness [136]. From the above-mentioned reports, it can be concluded that Ca^{2+} signals do play a significant role in plant responses to cold stress [100, 146].

Very recently, Nawaz *et al.* (2014) have reported that among the 16 CNGC genes found in rice, the expression level of 10 OsCNGCs was upregulated under cold stress [21]. It was observed that the expression of OsCNGCs belonging to phylogenetic group I, II and III was significantly upregulated by cold stress while members from group IV were down regulated. In summary, OsCNGC6 showed the highest expression level (192-folds increase), while OsCNGC16 showed the lowest expression (2-fold decrease) in response to cold stress. Although further studies for the expression validation based on gene knockout techniques are necessary to properly ascertain their functions, these results certainly indicate that OsCNGCs play discrete role in plant responses towards cold stress [21] (Fig. **1**).

CNGCb gene from the plant species *Physcomitrella patens* and its Arabidopsis ortholog CNGC2 is believed to encode a component of cyclic nucleotide gated Ca^{2+} channels that function as a primary thermosensors of land plant, deciphering a mechanistic basis for heat-induced cytoplasmic Ca^{2+} concentration increment. It is speculated that CNGC2 and CNGCb form heteromeric Ca^{2+} channels with other related CNGCs [39]. These plasma membrane localized channels respond to increase in the ambient temperatures by ensuing an optimal heat shock response, which imparts acquired thermotolerance in plants [39]. Furthermore, AtCNGC6 is also involved in mediating heat induced Ca^{2+} influx and thereby triggers expression of heat shock protein (HSP) genes and development of acquired thermotolerance in plants [41].

4. Role of CNGCs in Plant Nutrition and Calcium Homeostasis

Ion homeostasis in plants depends compulsorily on the fine-tuning of membrane transporter proteins and a disruption of the ionic equilibrium can have deleterious effects on plant growth and development. The Arabidopsis plants expressing AtCNGC10 antisense transcripts showed altered growth, starch accumulation and response to salt stress in the transgenic plants [42, 147] while null mutations in CNGC2 caused Ca^{2+} hypersensitive dwarf phenotype probably due to some defect in Ca^{2+} related sensing or signaling mechanism [148]. Owing to similar physicochemical properties with Ca^{2+} and inability of CNG channels to discriminate between different cations, the channels have been shown to allow entry of toxic heavy metals such as Ni^{2+}, Sr^{2+} and Pb^{2+}. NtCBP4, a close tobacco homolog of AtCNGC1 shares 74% identity at the peptide level and NtCBP4 overexpressing transgenic plants show an increased tolerance to Ni^{2+} and a hypersensitive phenotype to Pb^{2+} [21]. This finding corroborates well with the enhanced

tolerance of atcngc1 T-DNA mutants to Pb^{2+} or transgenic plants expressing a truncated, inactive version of NtCBP4 [149]. In a study on Arabidopsis quantitative trait loci involved in Cesium (Cs^{+}) and Strontium (Sr^{2+}) accumulation, Kanter *et al.* (2010) could show that the *AtCNGC1*, 3, 9, 11, 12 and 17 are among the potential candidate genes involved in the natural variation of Sr^{2+} accumulation [150].

Studies on the cation absorption characteristics done on intact barley roots have shown that plants have evolved at least two distinct mechanisms of K^{+} uptake: a high-affinity mechanism that preferably transports K^{+} over Na^{+} and a low-affinity mechanism that transports both the monovalent cations [151, 152]. Regulation of intracellular K^{+} homeostasis is crucial to mediate plant adaptive responses to various abiotic and biotic stresses including salinity, drought and oxidative stress [153]. Loss-of-function studies have revealed that at least three CNGCs namely AtCNGC1, AtCNGC3 and AtCNGC10 contribute in nonselective monovalent cation uptake in Arabidopsis. The T-DNA insertion mutant of *Atcngc1* accumulates less Ca^{2+} and K^{+} in shoot part of plants than wild type, and shows less sensitivity to toxic concentrations of Na^{+} [38, 110, 152, 154] implying that AtCNGC1 is capable of transporting these cations needed for various physiological functions. Like AtCNGC1, *Atcngc3* mutants are also less sensitive to growth-impeding concentrations of K^{+} and accumulate less K^{+} than wild type [40]. As the germination of *Atcngc3* mutant seeds is significantly lower than the wild type on higher NaCl concentration, this suggested that the mutant embryos are hypersensitive to salt stress. Moreover, this lead to the speculation that in developing embryos AtCNGC3 is perhaps playing role in mitigating the ionic toxicity by facilitating the movement of Na^{+} from salt-sensitive to salt-tolerant tissues [40]. A possible role of CNGC in boron nutrition stress may also be speculated as boron starvation invokes the expression of AtCNGC19 in roots [155].

5. Role of CNGCs in Response to Pathogens

Previous studies [156] have indicated that inoculation of leaves with a pathogen (*Pseudomonas syringae*) leads to increase in the cytosolic Ca^{2+} of plant cell. When the leaves were exposed to a Ca^{2+} channel blocker, it prevented hypersensitive response (HR) in wild type plants inoculated with pathogen. This result supported the concept that pathogen-associated molecular pattern (PAMP)-related Ca^{2+} influx is an early signal, which is initiated in plants in response to pathogen attacks. Loss-of-function study with many Arabidopsis CNGCs has revealed that there is an altered plant response to pathogens. Loss-of-function mutation in *CNGC2* (resulting "defense-no-death" or *dnd1* mutant) and *CNGC4* (resulting *dnd2* mutant) caused altered plant responses to avirulent pathogens. Arabidopsis plants with these mutations exhibit impaired HR, constitutive expression of salicylic acid (SA) as well as changes in the expression pattern of pathogen defense related genes. Another proof of CNGC function in plants against pathogen defense came in the form of *cngc11/12* chimera mutant. Yoshioka *et al.* (2001) reported that this mutant called constitutive expressor of PR gene 22 (cpr22), harbors a 3 kb genomic deletion that joined *AtCNGC11* with *AtCNGC12*, thus creating a chimeric CNGC [157]. The *cpr22* mutant displays altered defense responses, stunted growth and curly leaves. Detailed investigation of *cngc11* and *cngc12* mutants individually revealed a higher degree of susceptibility to *H. parasitica* (EMWA1) compared to wild type while no differences were observed in their morphology, PR gene expression or lesion formation [21]. When the two mutants were self-crossed, the F1 progeny displayed a phenotype similar to their parents. These results proved that the *cpr22* phenotype is not because of loss of one of these genes or due to *CNGC11/cngc11*, *CNGC12/cngc12* genotype, but is rather a result of the *AtCNGC11/12* chimera formation [21]. Therefore, these findings lead to infer that CNGCs are involved in plant defense against pathogens in both dicotyledon and monocotyledon plants. Detail studies with the dnd1 mutant have provided some interesting insight into the specific mechanisms underlying the role of CNGC-mediated cytosolic Ca^{2+} increase in pathogen elicited signaling [21, 49]. In this work, when *dnd1* plants were supplied with an exogenous nitric oxide (NO) donor, HR was restored as generation of NO is essential for HR development. Damage associated molecular pattern (DAMP) and/or Pathogen associated molecular pattern (PAMP)-mediated by LPS-lipopolysaccharide results in cytosolic Ca^{2+} elevation and CNGC-dependent NO production. Therefore, it was inferred that CNGC-dependent cytosolic Ca^{2+} increase is involved in the pathogen/PAMP-elicited NO production, which in turn, in the presence of a suitable *avr* gene in the pathogen and a counterpart R gene in the plant, leads to HR response. Growing body of evidence indicate that the Ca^{2+} binding proteins like CaM, or a CaM-like proteins (CML) regulates the above mentioned NO production downstream from CNGC-mediated cytosolic Ca^{2+} increase during defense signaling [14]. It is believed that CaM or CML is involved in the mechanism to transduce CNGC-dependent cytosolic Ca^{2+} increase upon pathogen perception (Fig. **2**).

Besides mediating some of the downstream signaling from the early PAMP/pathogen-elicited cytosolic Ca^{2+} increase, the increase in the CaM activity due to CNGC mediated Ca^{2+} influx could also shape the Ca^{2+} signal [64]. Further work has also begun to provide more insights into the CNGC-dependent Ca^{2+} permeability into the cytosol that could impact the expression of defense-related genes during pathogen response signaling. One of such example is *Arabidopsis CBP60g* gene product, which is responsive to CaM protein. This gene product is crucial for PAMP-mediated SA production and defense related processes [33, 158]. The importance of the CBP60g protein could be understood from the fact that absence of CaM interaction with this protein results in enhanced disease progression.

CONCLUSION AND FUTURE PROSPECTS

Abiotic and biotic stresses have been recognized as a major threat to the agricultural system. To cope up with these adverse effects, plants induce several physiological processes and molecular mechanisms. Investigations have shown Ca^{2+} channels as strong and potential tool in alleviating the non-conducive effects of biotic and abiotic stresses in plants. Ca^{2+} channels play pivotal roles in diverse array of cellular processes. Although functions of many Ca^{2+} channels in plants have been unraveled but their regulatory

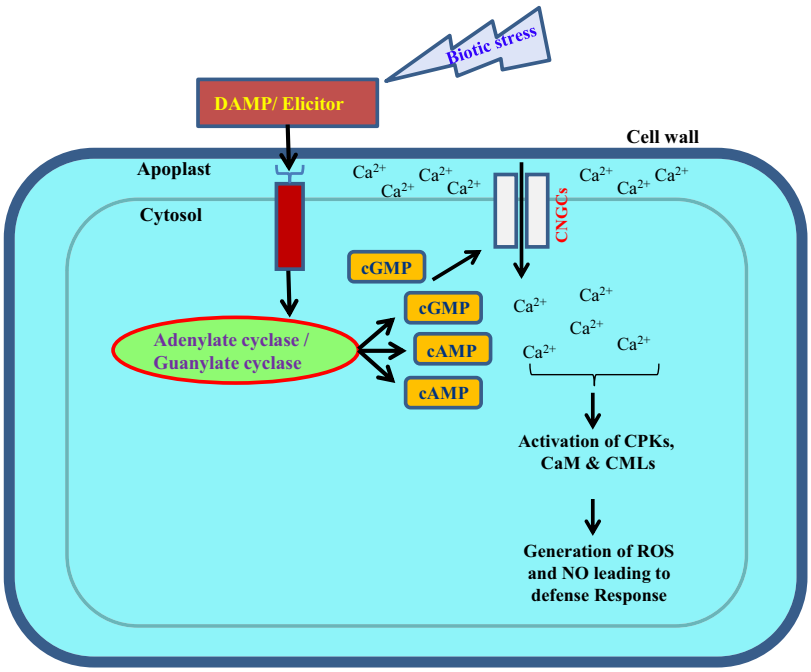

Fig. (2). Model depicting defense signaling cascade generated in response to biotic stress. (1) Damage-associated molecular pattern (e.g. endogenous plant elicitor peptides-Peps) binding to PEP receptors (PEPRs) increases cytosolic cGMP which activates the Ca^{2+}-permeable CNGC2 to facilitate Ca^{2+} entry into the cytosol. (2) CNGC2-mediated cytosolic Ca^{2+} elevation results Ca^{2+} binding to CPKs (calcium dependent protein kinases), CaM (calmodulin) and CML (calmodulin-like proteins) and their activation result in heightened expression of defense genes and activation of *RbohD* and *RbohF* which are involved in ROS (reactive oxygen species) and NO (nitric oxide) generation. (3) ROS and NO can act as antimicrobial compound and limit the growth of a virulent bacterial pathogen in a plant [159].

mechanisms at the molecular level remain largely elusive. As Ca^{2+} channels in plants belong to large gene families, there seem to be functional redundancy among them. To solve this puzzle generation of multiple knockout mutants and/or simultaneous knockdown by the use of artificial microRNAs may be attempted to address the functional redundancy issue and gain more insights into the functions of Ca^{2+} channels in *planta*. In addition to these, identification of interacting partners and regulatory molecules of Ca^{2+} channel proteins *in vivo* utilizing a systemic approach would help to decipher the regulatory mechanism, gene expression and cellular signaling networks that involve Ca^{2+} channels. The recent findings regarding the possible involvement of plant CNGCs described in this review and the expression analysis together with the identification of interacting partners (though only few have been identified) bear witness to this dynamic research field.

Plant CNGCs gene functions have been implicated in diverse aspects of plant growth and development including responses to hormonal, biotic and abiotic stimuli (Table **1**). Previously, many mutants of CNGCs, and transgenic plants expressing full-length or mutant CNGCs, have been characterized to investigate their role in plants. In brief, AtCNGC1 is probably involved in Ca^{2+} uptake in plants, AtCNGC2, 4, 11 and 12 in plant disease and defense signaling pathway. Role of AtCNGC3 is speculated to be in cellular homeostasis and AtCNGC2, 4, 7, 8, 10, 16 and 18 in plant growth and development. Plant CNGCs display diverse temporal and spatial distributions, voltage-dependence, cation conductance, and regulation by CaM, CML and kinases. Studies conducted with loss-of-function mutants suggest that at least three CNGCs are involved in nonselective uptake and transport of cations in Arabidopsis: AtCNGC1, 3 and 10. There are 20 CNGCs in Arabidopsis, 16 in rice and 18 in tomato. Several assumptions were put forward to understand why plants have adopted CNGCs for nutrient absorption and homeostasis. As plants are devoid of canonical voltage-gated Ca^{2+} channels, the purpose of this channel family is possibly to serve as a Ca^{2+} uptake route. Moreover, low-affinity transport systems, such as those represented by NSCCs (CNGCs belong to NSCCs), possess a greater influx capacity than highly selective, high-affinity transporters. This feature may prove to be advantageous in natural environments, where plants have to compete for limited resources, by allowing faster rate of cation absorption. Plants may also acquire Na^+ through CNGCs, though excessive Na^+ concentration may be fatal for plant life, moderate Na^+ levels can be helpful to the growth of many plant species under K^+ starvation conditions. The entry of toxic Pb^{2+} into root cells occurs at least partially via Ca^{2+}-permeable channels. AtCNGC1 protein is known to be predominantly located in roots and heterologous expression of AtCNGC1 channel has proved that it is an inward-rectifying channel for K^+, Na^+ and Ca^{2+}. Moreover, *Atcngc1* mutants showed a higher tolerance to Pb^{2+} and accumulated lower Pb^{2+}content than wild type plants. Interesting phenotype can be anticipated in the gain-of-function of AtCNGC1 in K^+ starvation condition. Additionally, as *Atcngc1* mutants accumulated lower amount of Pb^{2+} than wild type plants, future studies to explore their potential as a tool for detoxifying Pb^{2+} polluted soils can be expected.

There are still a few interesting questions awaiting further investigation. For example, presence of various *cis*-regulatory elements may be explored in the upstream sequences on both of the strands for their functional role. In addition, the *OsCNGC* genes transcripts significantly respond to multiple stimuli, as demonstrated by their expression patterns to exogenous hormonal (abscisic acid, indole acetic acid, kinetin and ethylene), biotic (*P. fuscovaginae* and Xoo) and abiotic (cold) stresses. This differential expression patterns provide a platform to further elucidate the functions of plant CNGCs. Unveiling intricacies of the complete CNGCs mediated Ca^{2+} signaling in biotic and abiotic stresses and the relationship between different regulatory molecules of CNGCs in plant immunity and abiotic stress responses would be fascinating and rewarding. Meanwhile, more genomics and proteomics studies outcomes are expected to reveal CNGCs mediated processes. Molecular dissection is also needed to gain insights into the CNGCs-mediated signaling in response to specific plant hormones/metabolites. Overall, finer details of CNGCs-mediated defense networks as well as further insights into the development of abiotic stress tolerance can be uncovered through adopting a comprehensive integrated approach incorporating genetics, molecular biology, biochemistry, genomics, proteomics, bioinformatics and computational biology.

LIST OF ABBREVIATIONS

CNGC	=	Cyclic nucleotide-gated channel
CaMBD	=	Calmodulin binding domain
cNMP	=	Cyclic nucleotide monophosphate
HR	=	Hypersensitive response
WUE	=	Water use efficiency
cNBD	=	Cyclic nucleotide binding domain

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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