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Role of G Protein Signaling Components in Plant Stress Management

Nisha Khatri[#] • Arpana Katiyar[#] • Yashwanti Mudgil[*]

Department of Botany, University of Delhi, Lab number 45, North Campus, Chatra Marg, Delhi 110007, India

Corresponding author: ***ymudgil@botany.du.ac.in, ymudgil@gmail.com**

[#] These authors contributed equally to this article

ABSTRACT

Plant growth and development is controlled by several environmental cues, including biotic and abiotic stresses. These changes are sensed through different cell surface receptors, which undergo conformational changes and transmit the signal downstream. These signals are transmitted via intracellular signaling molecules that ultimately modulate gene expression, which in turn helps them to survive the environmental challenge. One such class of receptors is plasma membrane localized G-protein-coupled receptors (GPCR). G proteins have been shown to transduce signals from GPCR. Plant G proteins play important role in both biotic and abiotic stresses. With the availability of G protein core complex component null mutants, it has been shown that G proteins play role in jasmonic acid (JA)-mediated response during infection by *Alternaria brassicola* and *Fusarium oxysporum*. While *Gα* null mutants are less susceptible to necrotrophic pathogen, G protein components have also been found to play a regulatory role in ethylene-mediated hypoxia signaling. Abiotic stress-generated signals also activate G-protein signaling cascade, *Gα* over expressing plants for instance, showed tolerance to high salinity and high temperature whereas over expression of *Gβ* showed tolerance to high temperature. *RGS1* (Regulator of G-protein Signaling1) over-expression confers drought tolerance via ABA mediated pathway by stimulating the expression of enzymes involved in biosynthesis of ABA. In plants, very few effectors of G protein signaling were known, until recently with the availability of G protein signaling interactome in *Arabidopsis*, proteins have been re-discovered as novel component of G protein signaling pathway. Some of these are plant homologs of animal proteins with known/predicted function in stress, but their role in plants is yet to be discovered. In this review, we focus on the involvement of G-protein signaling components during biotic and abiotic stress.

Keywords: abiotic stress, biotic stress and G protein interactome, G-protein couple receptor, heterotrimeric G-protein

Abbreviations: **ABA**, abscisic acid; **ABI1**, ABA insensitive; **BR**, brassinosteroid; **CDPK**, Ca^{2+} dependent protein kinase; **CESA**, cellulase synthase; **DAG**, diacylglycerol; **DGK**, DAG kinase; **ET**, ethylene; **GA**, gibberellin; **GPCR**, G-protein coupled receptor; **GTG**, GPCR-type G protein; **H_2O_2**, hydrogen peroxide; **HR**, hypersensitive response; **IP_3**, inositol 1,4,5-trisphosphate; **JA**, jasmonic acid; **MAPK**, mitogen-activated protein kinase; **NPC**, non-specific phospholipase; **PA**, phosphatidic acid; **PBZ1**, probenzole inducible protein; **PC**, phosphatidylcholine; **PI**, phosphoinositide; **PLD**, phospholipase D; **PLC**, phospholipase C; **PLA**, phospholipase A; **PP2C**, protein phosphatase 2C; **PR**, pathogenesis related; **RGS**, regulator of G-protein signaling; **ROS**, reactive oxygen species; **SA**, salicyclic acid; **SE**, sphingolipid elicitor; **Y2H**, yeast two hybrid

CONTENTS

INTRODUCTION

Being sessile, rooted and immobile plants are exposed to unfavorable environmental conditions; therefore adaption of plants with constantly changing external environment is very important for their survival. Intracellular cell signaling is the link that connects plant's external environment with internal via a chain of events mediated by signaling molecules such as GPCR proteins. Signal perceived at membrane level leads to conformational change in intracellular

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domain of GPCR and which in turn activates associated G-protein and downstream signaling. Activated form of G-protein further act on downstream targets to generate a cascade, which finally leads to change in expression of the genes, which helps the plant to survive in adverse condition (Misra *et al*. 2007).

G protein signaling plays important role in diverse biological processes ranging from seed germination, seedling growth responses to light, phytohormones, ozone, sugars, pathogen resistance, etc. (Chen 2008b).

Heterotrimeric G proteins are found ubiquitously in all eukaryotes from yeast, slime molds to higher plants and mammals hence designated as a conserved component in signal transduction. Compared to animal systems, G-protein signaling core complex is much simpler in plants (Temple and Jones 2007). In *Arabidopsis* genome only one or two genes for each subunit have been found, only one canonical Gα subunit gene (*GPA1*) (Ma *et al*. 1990; Temple and Jones 2007), one canonical Gβ subunit gene (*AGB1*) (Weiss *et al*. 1994), and two Gγ subunit genes (*AGG1* and *AGG2*) (Mason and Botella 2000). Similar numbers of genes encoding G protein subunits have been reported from the monocot, rice (*Oryza sativa*) (Ma *et al*. 1990; Abe *et al*. 2012). In contrast, four Gα, four Gβ and ten Gγ subunit genes are described in legume, soybean (Kim *et al*. 1995; Bisht *et al*. 2010; Choudhury *et al*. 2011; Pandey 2011). Comparatively only few genes are found to encode G protein subunits in plants, which suggest that these available genes are sufficient for diverse signaling events.

GDP-bound form of the N-terminal helix and three 'switch' regions of Gα interacts with a seven bladed propeller structure in the β subunit (Morris and Malbon 1999). After perceiving signal from GPCR, Gα changes conformation in such a manner, which allow GTP binding, consequent reorientation of the switch regions in the Ras domain disrupts the tight interaction between Gα and Gβ, which results in separation of Gα from Gβ/Gγ dimer and Gα and/or Gβγ and subsequently these free subunits then interact with downstream effector molecules, which are either ion channels or enzymes. G-protein coupling to effectors results in generation of second messenger. Very little is known about these effectors in plants (Jones and Assmann 2004). A close look at the recently discovered complete G protein interactome in *Arabidopsis* (Klopffleisch *et al*. 2011) re-discovers stress related proteins as novel component of G protein signaling. These identifications have great significance for our current understanding of G protein signaling network in various kinds of stresses and to find out the complete pathway of particular stress response. In this review, we discuss role of G protein signaling components in abiotic and biotic stress, which would be helpful in devising stress management strategy in crops.

ROLE OF G PROTEIN AND INTERACTORS IN ABIOTIC STRESS

Crop production and plant growth are adversely affected by various kinds of abiotic stresses. Therefore, it is important to understand signal perception to particular stress followed by transmission effector molecules in the signaling cascade. Several key questions remain unanswered such as, what are the effectors molecules involved in response to specific kind of stress? Is there cross talk between various effectors involved in different kind of stresses? Lot of meaningful information need to be produced in plants so that signaling pathways can be manipulated for proper stress management strategies.

Plants undergo reprogramming to cope with various abiotic stresses so that they survive even under changing environment and understanding this reprogramming is the need of the hour. Major stresses encountered by plants in today's world are drought, elevated temperature, salt and elevated carbon dioxide. These external changes finally lead to change in transcriptome, proteome and metabolome (Kaplan *et al*. 2007; Kim *et al*. 2007; Shulaev *et al*. 2008).

G protein coupled receptors: stress sensor at the membrane

Different sensors have been expected for each kind of stress but none have been confirmed for cold, drought, or salinity as yet. All these three stresses have been shown to induce transient Ca^{2+}influx into the cell cytoplasm and stimulate the accumulation of compatible osmolytes and antioxidants. Therefore, undiscovered cell surface receptors perceiving these stress signals and leading to this Ca^{2+} influx may represent sensors for these stress signals (Hasegawa *et al*. 2000; Cheong *et al*. 2003; Batistic and Kudla 2012).

In yeast and in animals, mitogen-activated protein kinase (MAPK) pathways are responsible for the production of compatible osmolytes and antioxidants (Hasegawa *et al*. 2000). These MAPK pathways are activated by receptors/ sensors such as protein tyrosine kinases, G-protein-coupled receptors, and two-component histidine kinases (Urao *et al*. 1999; Taj *et al*. 2010; Sinha *et al*. 2011). Signal is transferred through membrane and best-characterized plasma membrane receptors known in plants are transmembrane receptors, usually RLK or receptor-like kinases such as At RLK3, Srlk, NtC7, RPK7, etc. (Nam *et al*. 1997; Czernic *et al*. 1999; Sano *et al.* 2003; Crespi *et al.* 2009). Although, approximately 400 putative divergent seven transmembrane receptors have been speculated in *Arabidopsis* but till date only a single gene encoding putative GPCR has been characterized in *Arabidopsis* (Colucci *et al*. 2002; Moriyama *et al*. 2006). Recently, putative GPCR has been isolated from *indica* rice and role of this GPCR in mediating abiotic stresses have been suggested (Yadav and Tuteja 2011). Expression profiling showed GPCR induction following NaCl and ABA treatments. However, drought leads to up-regulation of GPCR during early exposure followed by decrease in its transcript level. No significant change in transcript was detected due to cold and heat stress. These findings predict direct regulation/involvement of rice GPCR under abiotic stress conditions (Yadav and Tuteja 2011). The molecular machinery underlying these receptors is described in the following section.

Gα subunit and effectors

In plants stress signals received at membrane by receptors are relayed further in cell that either leads to activation of ion channels or activates enzymes like phospholipases. Phospholipids are the major component of cell membrane and are involved in signaling through membrane specific catabolism by phospholipases. Phospholipases hydrolyzes phospholipids into fatty acids and other lipophilic substances and can be classified as Phospholipase A (PLA), Phospholipase C (PLC) and Phospholipase D (PLD); depending upon the site of cleavage. These phospholipases play critical roles in both biotic as well as abiotic stresses by releasing different second messengers or by activating G protein heterotrimer and downstream signaling events.

Phospholipase C

PLCs are classified into three subfamilies namely γ (gamma), β (beta) and δ (delta) with catalytic properties of all three PLCs being similar. IP_3 and DAG are the two products generated by PLC that act as second messengers. Mammalian IP_3 releases $Ca^{2+}$from intracellular stores whereas DAG activates certain other members of the PKC super-family. DAG is rapidly phosphorylated by DAG kinase (DGK) to PA (Testerink and Munnik 2011). None of the PKCs have been discovered from plants and may be considered absent. Abiotic stress conditions like oxidative stress, ABA treatment and wounding mainly activates PLD whereas biotic stress like pathogen elicitors activates PLC-DGK (Arisz *et al*. 2009).

Based on the sequence information from various plant species, plants seem to have only delta subfamily of PLC. Plant PLC δs have two subcategories one that uses phos-

phoinositide as substrate, the PI-PLCs of which, there are 9 known in *Arabidopsis* (Mueller-Roeber and Pical 2002). The second category of PLC hydrolyses structural phospholipids, termed NPCs (for non-specific PLCs). Six NPC genes are present in *Arabidopsis* designated as NPC1-NPC6 (Testerink and Munnik 2011).

The *AtPLCl* transcript level is strongly induced by salt and drought and slightly by cold (Shinozaki and Yamaguchi-Shinozaki 1997). PLC transcripts are up-regulated during drought and salt stress in tobacco and results into tolerance against high level of drought and salinity (Tripathy *et al*. 2012). Involvement of PLCs was also investigated in regulation of proline accumulation in *Thelungiella salsuginea*. It was observed that inhibitor of PLC slightly inhibits proline accumulation in high salt and mannitol-stressed seedlings and externally supplied calcium reversed the inhibitory effect of aminosteroid PLC inhibitor U73122, showing involvement of PLC via calcium signaling (Ghars *et al*. 2012). In *Zea mays*, PI-PLC also is up regulated in roots during drought stress. Transgenic gain-of-function approach showed higher relative water content, better osmotic adjustment, increased photosynthesis rates, lower percentage of ion leakage and less lipid membrane peroxidation, and resulted in higher grain yield than the WT; and those expressing the antisense transgene exhibited inferior characters as compared to the WT, clearly showing its involvement in drought stress (Wang *et al*. 2008). GPCR transduces signals to Heterotrimeric G-protein, after propagation of signal GDP which is associated with Gα subunit get exchanged with GTP and as a consequence of this Gβγ dimer get dissociated from Gα subunit and this activated Gα interacts with and regulates PLC.

PLC from *Pisum sativum* (PsPLC) interacts with PsGα and PsGβ *in planta* as well as *in vitro* (Misra *et al*. 2007). Both PsGα1 and PsGβ are induced by heat, salinity and hydrogen peroxide stresses in pea. Gα mediated pathway confers tolerance during salinity and high temperature stresses whereas Gβ provides tolerance to heat stress. The role of calcium during salinity stress sensing through various mechanisms has been elucidated, and thus one may conjecture PLC mediated pathway is involved in conferring tolerance towards salinity (Misra *et al*. 2007). Using various inhibitors and activators of G protein signaling components, it was found that during early stage of salt stress response signal is transduced from G-protein to GMK1 (*Glycine max* MAP kinase 1) via PLC and PLD. Mastoparan, an activator of G-protein also activates GMK1 and inhibitors of PLC and PLD inhibits the activity of GMK1 and also activity is reduced when G-protein and phosphotidic acid (PA) inhibitors were added, this clearly showed that GMK1, G-protein and phospholipases interact during salt stress (Im *et al*. 2012).

Structural phospholipids, which are hydrolysed by NPCs, are also involved in stress responses. Expression of *Arabidopsis* NPC isoform, NPC4, is highly induced by salt. Knockout mutant lines showed increased sensitivity to salt stress and water deficiency, decreased ABA sensitivity in seed germination, root elongation, and stomatal movement (Kocourkova *et al*. 2011) NPC4 over expression imparted higher sensitivity to ABA and tolerance to hyperosmotic stress. Recent studies suggest that NPC4 produced DAG is converted to PA and other derived lipids positively modulate ABA and promote salt and drought tolerance (Peters *et al*. 2010).

Phospholipase D (PLD)

One of the well characterized effector of Gα subunit is PLD (Wang 2002). PLD hydrolyzes membrane lipids to generate PA and has been shown to play role in abscisic acid (ABA) signaling, programmed cell death, conferring freezing tolerance, and other stress responses (Sang *et al*. 2001b; Hong *et al*. 2010). Twelve *PLD* genes are reported from *Arabidopsis* that can be divided into two subfamilies, C2-PLD and PX/PH-PLDs. C2 is a Ca^{2+} and phospholipid-binding domain, and the PX and PH domains refer to two distinct phosphoinositide-interacting structural folds, with phox homology and pleckstrin homology, respectively (Elias *et al*. 2002; Qin and Wang 2002).

Using mutated protein and immune-precipitation, PLDα1 was found to be interacting with Gα subunit. Binding takes place through a sequence motif analogous to the DRY motif normally conserved in animal G-protein-coupled receptors. Interestingly, this interaction prefers GDP state of Gα and GTP state is found to be inhibitory for the interaction. The physiological significance of this interaction has been recently indicated in stress condition (Zhao and Wang 2004). Mutation of amino acid residues in DRY motif abolishes the PLD*α*1-G*α* binding. *Arabidopsis* deficient in PLD*α*1 displays alterations in various plant processes, such as reactive oxygen production, wound-induced accumulation of jasmonic acid, freezing tolerance, water loss, and abscisic acid signaling (Wang 2002). PLD*α*1-G*α* interaction also stimulates the intrinsic GTPase activity of G*α*. These results indicate that the interaction reciprocally modulates the activities of PLD*α*1 and G*α*. PLD hydrolyzed PA binds to ABA insensitive (ABI1), a protein phosphatase 2C (PP2C) that is a negative regulator of ABA responses in *Arabidopsis*. The PA binding decreases PP2C activity and also appears to reduce its translocation to nuclei in response to ABA by tethering ABI1 to the plasma membrane (**Fig. 1**). These results show that activation of PLD*α*1 inhibits the function of the negative regulator ABI1, thus promoting ABA signaling (Zhang *et al*. 2004). Antisense suppression of *PLDα1* shows several fold higher tolerance to freezing stress, and lipid profiling shows that PLD*α*1 activity results in a significant decrease in phosphatidylcholine (PC) and an increase in PA (Welti *et al*. 2002). Depletion of PLDα shows less sensitivity to ABA and hence impaired stomata closure during water stress and, as expected, PLDα over-expressed plants shows enhanced sensitivity to ABA in leaves (Sang *et al*. 2001b).

*PLD*δ have properties that are different than other PLDs. Expression of *PLDδ* increases under severe dehydration, high salt, and during cold acclimation. *PLDδ*-null mutant plants are less tolerant to freezing, whereas over-expression of *PLDδ* increases freezing tolerance in *Arabidopsis*. Lipid profiling indicates that PLD*δ* activity produces selective PA species but does not result in substantial lipid hydrolysis during freezing stress (Li *et al*. 2004). Both *PLD*δ *and PLDα1* play different roles under freezing stress. Interestingly, the change in *PLDδ* level in plant do not result in changes in the expression of the cold-regulated genes *COR47* or *COR78*, moreover the increase in the levels of compatible osmolytes, proline, or soluble sugars have not been reported, which are known to play a role in plant freezing tolerance. This suggests that *PLD*δ follows some yet to be discovered pathway to confer cold tolerance (Li *et al*. 2004).

The *pld*δ mutant plants with functional PLDα showed significant repression in accumulation of PA during drought stress, clearly showing role of PLDδ and no involvement of PLDα in PA accumulation during drought stress (Katagiri *et al*. 2001). The most predominant form of PLD- PLDα, when suppressed showed reduced production of superoxide and exogenous application of PA restored superoxide formation to normal levels, which depicted the role of PLDα in production of superoxide via PA production (Sang *et al*. 2001a).

Concentration of Reactive Oxygen Species (ROS), hydrogen peroxide (H_2O_2) increases under various conditions including freezing stress. PLD*δ* is activated by H_2O_2 and plays a positive role by decreasing H_2O_2-promoted programmed cell death, this effect is mediated by PLD*δ* produced PA (Zhang *et al*. 2005). Recent whole genome transcript profiling of rice *PLD* revealed that a subset expresses differentially and significantly under specific or multiple abiotic stress conditions. A total of six *PLDs* were up-regulated, out of which four belong to α subfamily. This suggests α subfamily of *PLDs* has functional conservation

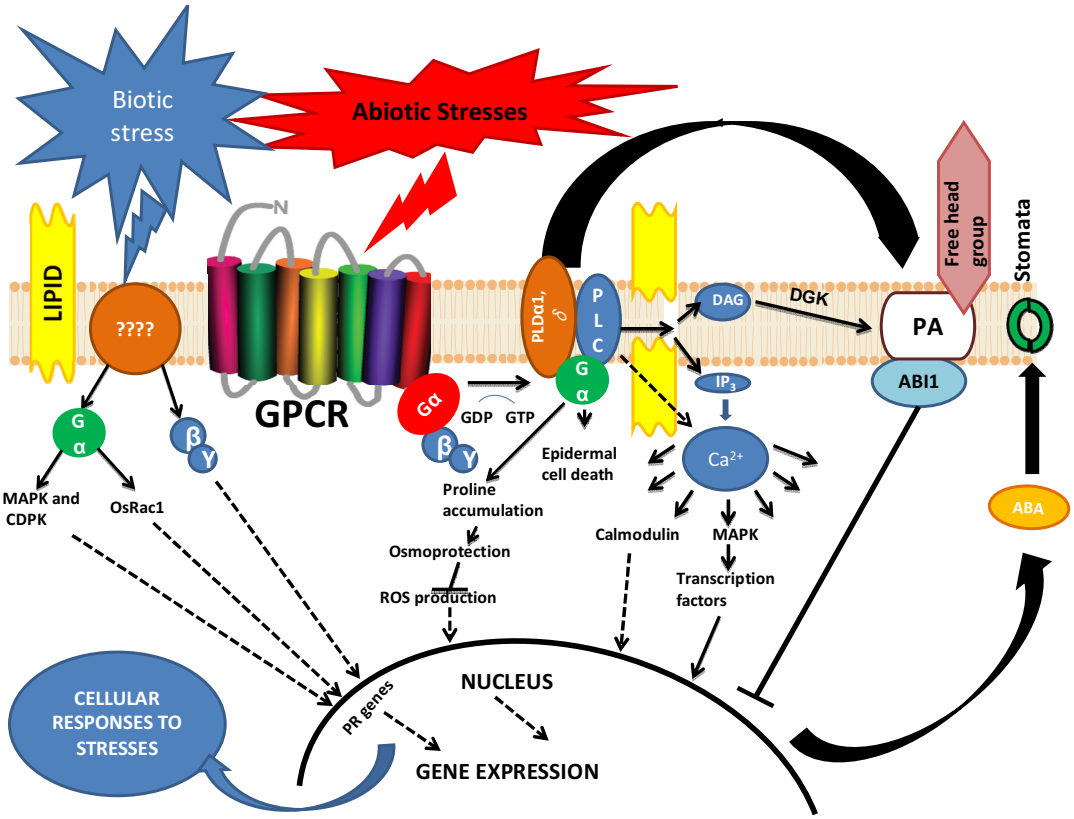

Fig. 1 Activity of G-protein is regulated by binding and hydrolysis of GTP to Gα subunit (Red colored Gα is showing inactive state and green colored Gα is showing activated form). PLDα1 (Phospholipase D) interacts with activated Gα subunit, PLD hydrolyzes membrane lipids to generate PA (phosphatidic acid) and free head group, PA binds to ABI1 (ABA insensitive). PA binding decreases PP2C (protein phosphatase 2C) activity and also reduces its translocation to nuclei in response to ABA by tethering ABI1 to the plasma membrane and promotes ABA signaling. PLC leads to production of IP3 (inositol 1,4,5-trisphosphate) and DAG (diacylglycerol) by hydrolysis of membrane lipids. IP_3 binds to IP_3-gated calcium channels and released Ca^{2+} into the cytosol, DAG get converted into PA by the action of DGK (DAG Kinase). PLC (Phospholipase C) also regulates the production of proline during salt stress. Calcium channels activated by IP3 also regulates Calmodulin, MAPKs and ion channels. Released Gβγ also activates signaling pathways involved in abiotic stress but players of these pathways are still a mystery. The receptor molecule which perceives the biotic stress still needs to be identified (marked by ????). However, Gα and Gβ/γ subunits along with their downstream effectors regulate the expression of PR genes which are involved in defense response. Dozens of effectors of Gα (GPA1), Gβ/Gγ1, Gβ/Gγ2 are discovered but need further studies to decode complete signalling pathways. The dashed arrows represent putative pathways that have not been confirmed till now in plants and solid arrows represent confirmed effectors.

in abiotic stress management and has evolved together in rice and *Arabidopsis* (Singh *et al*. 2012).

G-alpha protein (Gα)

The Gα subunit of the single *Arabidopsis* heterotrimeric G protein is also involved in regulating stomata closure in response to ABA (Wang *et al*. 2001). The foremost thing that happens during drought stress is an increase in the level of ABA, regulating stomatal opening and closure. Its level increases during drought and G protein is also involved in overall mechanism to help plants to withstand severe water deficit conditions. *Arabidopsis* genome contains only one gene encoding a single protein whose amino acid sequence is highly similar to eukaryotic RGS protein designated as AtRGS1. AtRGS1 strongly interacts with Gα subunit and also has GTPase activity implying that it is involved in G protein signaling (Chen *et al*. 2003; Jones *et al*. 2011). Over expression analysis of *AtRGS1* shows that it is involved in ABA signaling during drought stress since plants are more tolerant to soil desiccations as compared to wild type plants (Chen *et al*. 2006).

Abscisic acid (ABA) plays a central role in both abiotic and biotic stress adaptation (Fujita *et al*. 2006). Cold, dehydration and salt stress induce the production of ABA (Zhu 2002). The search for an ABA receptor has produced multiple candidates, including GCR2, GTG1 and GTG2, CHLH and PYR/PYL/RCAR receptor family (Liu *et al*. 2007; Chen 2008a; Pandey *et al*. 2009; Klingler *et al*. 2010; Guo *et al*. 2011). G proteins also modulate signaling via ABA. GPA binds to GCR1, GTG1 and GTG2 and *Arabidopsis* mutant lacking both *GTG1* and *GTG2* exhibit ABA hyposensitivity (Pandey and Assmann 2004; Pandey *et al*. 2009). Role of *GPA1* in regulating stress responses further explored using iTRAQ proteomics approach revealed differential expression of stress and ABA-responsive proteins, which also exhibited GPA1-dependency. Furthermore, it was proposed that GPA1 integrate biosynthesis and signaling of ABA by regulating the level of an ABA metabolizing enzyme (Alvarez *et al*. 2011).

Although infrequent, but one of the very destructing threat to plant is flooding and water logging stress. It was observed that level of ethylene increased in submerged tissue not only because of poor gaseous exchange but also because of hypoxia-induced ethylene. G-protein also play crucial role during hypoxia induced signal transduction (Steffens and Sauter 2010). The most important anatomical change or escape responses in response to hypoxia are formation of aerenchyma, rapid shoot elongation and of adventitious roots. Hypoxia-induced aerenchyma formation has been shown to be controlled by ethylene and G proteins in maize (He *et al*. 1996; Drew *et al*. 2000).

Semi-aquatic plants like rice are naturally adapted to submerged habitat through formation of adventitious roots from nodes. The widely accepted Davis-Roberts pH-stat theory suggests that plants rely on ethanolic fermentation for metabolic energy during prolonged anoxia (Fagerstedt *et al*. 2008). This oxygen deprivation has shown to cause changes in calcium and magnesium homeostasis (Chorianopoulou and Bouranis 2008). Epidermal cells, which covers

the root primordial undergo ethylene induced programmed cell death. Downstream to ethylene, hydrogen peroxide act (H_2O_2) as effector molecule, plants protect themselves from oxidative injuries by producing antioxidants Apart from this various genetic and cellular changes occur under these conditions.

In this cascade G-protein acts downstream to H_2O_2 and role of G-protein in epidermal cell death was revealed using *rga1* mutants (Mergemann and Sauter 2000; Steffens and Sauter 2009).

Three allelic mutant lines of the Gα subunit of rice gene *RGA1* with reduced mRNA levels showed strong inhibition of epidermal cell death. Neither submergence, nor treatment with ethylene or with H_2O_2 resulted in significantly elevated epidermal cell death rates in the rice dwarf (*d1*) mutants lines, lacking a single copy of *Gα* gene indicating that a heterotrimeric G protein acts downstream of ethylene and H_2O_2 and acts as a positive regulator of cell death (Suharsono *et al*. 2002; Steffens and Sauter 2009).

Carrot Gα subunit occurs as single or double copy genes in carrot genome, and its expression analysis studies suggest roles in heat- and salt-induced responses of the plant (Asakura and Kurosaki 2007). A full length cDNA encoding putative *Gα* subunit from *Brassica napus*, *BnGA1*, has been shown to be involved in salt and drought stresses and is down regulated under heat and cold stresses, expression of *BnGA1* is also regulated by brassinosteroid (BR) (Gao *et al*. 2010). In *Arabidopsis* BR is known to regulate gibberellin (GA) sensitivity and may also regulate ABA sensitivity (Ullah *et al*. 2002); it is therefore possible that *BnGA1* plays significant role in stress response mediated via hormones.

Role of Gα/*Gβ* subunit

A large variety of abiotic stresses, including extremes of temperature, high intensity of light, water deficit and air pollutants, such as ozone elicit production of oxidative burst i.e. transient increase in reactive oxygen species (ROS) that are essential for activating stress and defense responses. Both *Gα* and *Gβ* play role in signaling and cell death synergistically and individually in oxidative stress response to ozone in *Arabidopsis.* The null mutants of genes encoding *Gα* and *Gβ* subunits show differential sensitivities to ozone damaged tissue. *Gα* mutant shows less damage whereas plants mutated in *Gβ* subunit shows more tissue damage from O_3 (Joo *et al*. 2005).

G protein signaling also play important role in salinity and high temperature stress. Pea *Gα* and *Gβ* are induced in response to high heat and salinity but over-expression of *Gβ* from pea in tobacco confers resistance against high heat only and not to salinity. This clearly implies that other pathways are also involved along with G-protein mediated pathway to impart tolerance against salinity (Misra *et al*. 2007).

Interestingly, pea *Gα* and *Gβ* transcripts are also shown to be up-regulated during hydrogen peroxide treatment (Misra *et al*. 2007). H_2O_2 act as signaling molecule and regulates expression of some genes encoding antioxidants (Shu-Hsien HUNG1 2005), cell rescue/defense protein and signaling proteins such as kinases and phosphatases (Kamata and Hirata 1999). Absence of *Gα* and *Gβ* subunits also results in increased level of oxidative damage (Joo *et al*. 2005).

Role of Gγ subunit

The Gγ subunit of the G-proteins is essential for its proper targeting at the plasma membrane and correct functioning (Choudhury *et al*. 2011). A full length cDNA encoding a putative G-protein γ subunit (Gγ) isolated from *B. napus* and designated as *BnGG2* shared high sequence homology with *Arabidopsis* Gγ. BnGG2 was found to be up-regulated during salt and drought stress and down-regulated during heat and cold stress but further investigation at biochemical level are still awaited (Gao *et al*. 2010). Similarly, two isoforms of Gγ-*RGG1*(I) and *RGG2*(I) from rice (*Oryza sativa* cv *indica* group Swarna) show variation in transcript accumulation in response to abiotic stresses. Transcript level of both were up-regulated when plants were treated with NaCl, cold, heat and ABA; but during drought stress only *RGG1*(I) was up-regulated and stress related *cis*-regulatory motif was present in promoter of *RGG1*(I) clearly showed its involvement in stress (Yadav *et al*. 2012). Not much is known about Gγ subunit involved in stress and further detailed in-depth studies need to be executed.

Emerging role of G protein core complex interactome

It has been shown in animals that G protein core complex relay signal intra-cellularly with the help of downstream effectors or secondary messengers. In plants, very few candidates have been established as G protein effectors. Plant homolog of animal NDRG (N-MYC DOWNREGULATED REGULATED GENE) proteins have been re-discovered as novel component of G protein signaling (designated NDL1:N-MYC DOWNSTREAM REGULATED LIKE1) in *Arabidopsis* (Mudgil *et al*. 2009; Melotte *et al*. 2010). Although the precise molecular functions of NDRG/NDL proteins are unclear in plants, in animals these proteins play protective role in hypoxia and expression is induced by hypoxia and nickel, indicating its role in stress (Zhou *et al*. 1998; Roh *et al*. 2005). They may also play the similar role in plants stress signaling, which is yet to be discovered.

To identify plant G-protein effectors and scaffold proteins, detailed comprehensive analysis has been done in *Arabidopsis*. Exhaustive screening using a set of proteins from the G-protein complex using two-hybrid complementation in yeast was performed. Total 544 interactions were detected between 434 proteins, of which 68 highly interconnected proteins form the core G-protein interactome. This report highlights novel role for G-proteins in regulating cell wall modification (Klopffleisch *et al*. 2011). Our search for proteins having established or predicted function in abiotic and biotic stress in G protein signaling interactome database showed 40% (170 proteins out of 434) proteins have predicted function in stress response and majority of these stress related effector indicate function in abiotic stress (94% for abiotic stress and 6% for biotic stress).

More recent report about Pea G*β* interacting proteins using yeast two-hybrid (Y2H) approach hinted at their putative role in stress and development (Bhardwaj *et al.* 2012). These partners include thioredoxin H, histidine-containing phosphotransfer protein5-like, pathogenesis-related protein, glucan endo-β-1, 3-glucosidase (acidic isoform), glycine rich RNA binding protein, cold and drought-regulated protein (*corA* gene) and soluble inorganic pyrophosphatase1, suggesting the role of pea Gβ subunit in biotic and abiotic stress signal transduction and development (Bhardwaj *et al*. 2012).

ROLE OF G-PROTEIN DURING BIOTIC STRESS

A process of molecular communication is set in motion when a pathogen attempts to parasitize through physic-chemical interaction. Heterotrimeric G proteins are known to play role in this process of molecular communication. It was found that after the perception of the signal by GPCR there is an activation of G-protein, which further communicates with the other defense response pathways, like activation of PR (pathogenesis related) genes, or by an increase in the amount of some secondary metabolites. There is evidence that also suggests that the secondary metabolites and G-proteins follow the different resistance pathway (Trusov *et al*. 2009). Most of the studies have been performed using representative plants of the group i.e. rice and *Arabidopsis* and various mutants like: rice *Gα* dwarf*d1*mutants, *Arabidopsis* single *Gα* and *Gβ* deficient mutants, *Arabidop-*

sis agb1 mutants and *agg1agg2* double mutants (Suharsono *et al*. 2002; Komatsu 2004; Trusov *et al*. 2006; Delgado-Cerezo *et al*. 2011), *Arabidopsis* auxin signaling mutants, *axr1axr2* and *axr6* deficient in auxin stimulated SCF (Skp1-Cullin-F box) ubiquitination pathway (Llorente *et al*. 2008). Studies suggest that there is difference in the signaling behavior of monocot and dicot plants during biotic stress.

Signaling in rice

The first concrete genetic evidence for involvement of heterotrimeric G-proteins in defense mechanisms came from the research done in rice mutant *dwarf1(d1)*, which lack a functional *Gα* gene*, RGA1* and its interaction with the rice blast fungus, *Magnaporthe oryzae* (Suharsono *et al*. 2002). Inoculation of *d1* mutants with an avirulent race of *M. oryzae* or treatment with a sphingolipid elicitor (SE) resulted in induced expression of *RGA1*, highly reduced defense responses, including diminished ROS production, lower accumulation of pathogenesis related gene *PR1* and *PBZ1*, as well as reduced HR mediated cell death (Suharsono *et al*. 2002).

Likewise in response to virulent rice blight bacteria, *Xanthomonas oryzae* pv. *oryzae* (Xoo), *d1* plants developed earlier and more severe blight symptoms and showed delayed accumulation of defense proteins, and frequent wilting, suggesting an involvement of heterotrimeric G Protein also in defense response to virulent pathogens. *d1* mutants with a virulent strain of *M. oryzae* caused disease symptoms that were indistinguishable from wild type. These results implicate the heterotrimeric G Protein α subunit as an important player in rice resistance to bacterial and fungal pathogens (Suharsono *et al*. 2002).

Metabolite of probenazole (plant disease-defense systems activator) enhances the activation of a plasma membrane GTPase in response to a proteoglucomannan elicitor from the cell wall of blast fungus. A probenazole-inducible protein (PBZ1) was detected in wild type, but not in the *d1* mutant. After treatment with probenazol, PBZ1 reached maximal levels at 72 h in the wild type but maximal level was attained at 96 h in the *d1* mutant (Komatsu 2004).

During signaling mechanism, it was hypothesized that the signal from probenazole is likely to be received by an unknown receptor, and these signals may then be transmitted to the Gα protein. Gα is required for the phosphorylation of the 48-kDa putative MAPK and 55-kDa putative CDPK. The activated 48-kDa putative MAPK and 55-kDa putative CDPK, through a specific signal cascade, induce the production of PBZ1 protein and other defense-related reactions. The 48-kDa putative MAPK signaling pathway for probenazole is more important in resistance to bacterial blight than to blast fungus (Komatsu 2004).

Previous studies in tomato indicate that the heterotrimeric G protein may participate in plant defense signaling by increasing cytosolic Ca^{2+} in the cell (Aharon *et al*. 1998).

In defense signaling in rice, *Gα* plays an important role upstream of the small GTPase *OsRac1*. An interesting study suggested a model for the defense signaling of rice involving two different GTPases, the heterotrimeric G protein and the small GTPase OsRac1. *Gα* increases *OsRac1* mRNA abundance in the presence of SE. This may be one of the mechanisms to link the upstream *G* protein and the downstream *OsRac1* in the defense signaling in rice (Suharsono *et al*. 2002).

Signals from pathogens or elicitors such as SE are recognized by unknown receptors and transmitted to the heterotrimeric G-protein. *Gα* mRNA accumulation is induced by signals from receptors, and *Gα* is required for accumulation of *OsRac1* mRNA and induction of *PBZ1* mRNA expression. In the case of rice blast, transmission of signals is R gene-dependent. *Gα* transmits the signal to *OsRac1*, which has been shown to be a key molecular switch for the production of reactive oxygen species (ROS), defense-gene expression, and disease resistance (**Fig. 1**). Disease resistance is achieved by activation of multiple signaling pathways downstream of OsRac1 (Suharsono *et al*. 2002).

Signaling in *Arabidopsis*

The involvement of heterotrimeric G-proteins in *Arabidopsis* defense responses has been documented mainly for necrotropic pathogens. Mutants lacking a functional *Gβ* subunit, *AGB1*, showed increased susceptibility against the necrotropic fungi *Plectosphaerella cucumerina*, *Alternaria brassicicola* and *Fusarium oxysporum*, while *Gα*-deficient plants (*gpa1*) exhibited slightly enhance resistance to these pathogens (Trusov *et al*. 2006).

Resistance to these fungi in *Arabidopsis* is genetically complex and depends on the precise regulation of a large subset of signaling pathways, including those mediated by the hormones ethylene (ET), jasmonic acid (JA), salicylic acid (SA), ABA, and auxins (Robert-Seilaniantz *et al*. 2007). Resistance to necrotrophic and vascular fungi is also genetically determined by plant cell wall composition. Thus, *Arabidopsis* mutants impaired in cellulose synthase (CESA) subunits required for secondary (e.g. *irx*, irregular xylem) and primary (e.g. *prc1*/*ixr1*/*cev1*, procuste1/isoxaben resistant 1/constitutive expression of VSP1) cell wall formation showed enhanced resistance to different necrotrophic and vascular pathogens (Llorente *et al*. 2008). In *Arabidopsis*, cell wall integrity can activate specific defensive pathways, which could lead to plant enhanced resistance to different type of pathogens (Hernandez-Blanco *et al*. 2007).

In contrast to rice, defense-signaling pathway in *Arabidopsis* is mediated by *Gβ* subunit. During the defense responses, difference in the amount of some specific secondary metabolites in resistant plant compared with susceptible can be detected. So, it is hypothesized that the signaling cascade of heterotrimeric G-protein is somewhere related with increased concentration of secondary metabolite.

In downstream signaling mechanism, *Gβ* was found to interact with cytoplasmic proteins (Mudgil *et al*. 2009; Friedman *et al*. 2011; Klopffleisch *et al*. 2011). Recently, to draw a clear picture of AGB1 mediated downstream signaling, mutations were done at the surface residues of AGB1 to predict critical moieties for specific biological processes (Jiang *et al*. 2012). Different mutations affected different phenotypic responses, W109A fully restored silique morphology, flg22-triggered ROS production and fungal pathogen resistance (Jiang *et al*. 2012). In another study using similar approach Threonine residue at position 65 was found to be important in controling hypersensitivity to mannitol during seed germination (Chakravorty *et al*. 2012). These studies suggest divergent mechanisms of effector activation by G protein core complex components.

G-protein signaling is also involved in JA signaling pathway and the pathway of this jasmonate enhancement is different from plant's innate defense mechanism (Trusov *et al*. 2006). In mammalian system, Gβγ is required for the recruitment of Gα to the receptor and reactivation; plant deficient for *Gβ* not only affects the *Gβγ* but also *Gα* signaling mechanism (Clapham *et al*. 1993; Jones *et al*. 2004). Therefore, the increased resistance observed in the *Gα* mutant could be because of lack of the corresponding Gα-mediated signaling.

To check the above hypothesis, double mutants lacking both *Gα* as well as *Gβ* subunit had been generated and tested, symptoms of double mutants were closely related with *Gβ* mutants compared to wild type and *Gα* mutants. This strongly supports the predominance of *Gβγ* mediated signaling pathway during defense mechanism in *Arabidopsis* (Trusov *et al*. 2007).

AGB1 mutants display reduced induction levels of defense-related genes upon *A. brassicicola* infection. *Gβ* deficient mutants are more susceptible to infection with the necrotrophic pathogens, *A. brassicicola* and *F. oxysporum*, while *Gα* deficient mutants are less susceptible to the disease than wild type (Hernandez-Blanco *et al*. 2007). In *Arabidopsis*, there are only two genes coding for *Gγ* (*AGG1*

and *AGG2*), raising the possibility that the two potential G protein complexes mediate different cellular processes, and *agg1* mutants but not *agg2* mutants showed impaired resistance against necrotrophic pathogens (Trusov *et al*. 2007).

Both *agg1* and *agg 2* mutants showed hypersensitivity reaction to auxin-mediated lateral roots induction, suggesting that Gβγ1 and Gβγ2 synergistically inhibit auxin-dependent lateral root initiation (Trusov *et al*. 2007). Studies indicate a role of heterotrimeric G-proteins in plant defense in both monocotyledonous as well as in dicotyledonous plants. Interestingly, rice and *Arabidopsis Gα* deficient mutant displayed opposite pathogen responses. In *Arabidopsis*, the lack of the *Gα* subunit caused rather increased resistance to fungal pathogen, while in rice mutant exhibited reduced defense responses. Mutations in the *Gα* subunit induced different morphological phenotypes in both plant species, leading to dwarfism in rice, while in *Arabidopsis* the mutation produced rather the opposite effect, with mutants being slightly larger than wild type (Trusov *et al*. 2006). These differences suggest that the G-protein subunits could have functionally diverged during evolution in monocot and dicots. These studies also indicate that in both plant species, the extent of heterotrimeric G-protein signaling response to virulent and virulent pathogens is pathogen and/or elicitor dependent.

G PROTEIN SIGNALING IN STRESS

G-proteins play regulatory roles in multiple developmental processes ranging from seed germination, early seedling development to root development and organ shape determination (Chen 2008b), and also plays major role in stress signaling, in various type of stresses including biotic and abiotic (**Fig. 1**).

G protein subunits and phospholipases (PLCs and PLDs) are also involved in cascade and their expression change during severe dehydration, high salt, and during cold acclimation and ABA signaling. Environmental pollutants, ozone, low oxygen, water logging and oxidative burst also activate G-protein signaling, which also suggest its diverse function in plant.

During biotic stress, two pathways are found in monocot and dicot. In monocots, experiments on rice suggest that Gα subunit is involved in the downstream signaling mechanism during defense response. In dicots, Gβγ dimer plays the role in transmitting signals. Gβγ1 dimer is involved in necrotropic resistance in *Arabidopsis*. During the defense mechanism, various changes are found inside the plant system like changes in cell wall composition, enhancement of some specific secondary metabolites involving G-protein. Although the complete pathways are yet to be discovered but studies till now using mutants of rice and *Arabidopsis* shows that there is a direct relation between heterotrimeric G-protein and defense system of plant.

G-proteins have been found to transduce signaling during various kinds of abiotic stresses, these pathways are complex and require further detailed investigations.

The results of the various studies demonstrate the highly complex nature of G protein mediated plant adaptation to various stresses. Many approaches such as genetic studies, loss-of-function have made the picture somewhat clearer depicting the role of G-protein in stress signaling, but the connection among different effectors still needs to be established in signaling pathway. Approaches like recent G protein interactome in *Arabidopsis* are a step in that direction. Transcriptomic studies are well advanced but knowledge at proteome level is still limited and need to be explored.

A large number of genes identified in different studies using protein-protein interaction approaches have been currently annotated as genes with putative / or unknown function in plants and indicates that our knowledge is limited. This information is necessary for our understanding of the complex network of molecular changes that are important for stress tolerance in crop plants. Focused approach combining molecular, physiological and metabolic aspects of stress tolerance is required for bridging the knowledge gaps between the molecular or cellular expression of the putative effector genes and the whole plant physiology under stress. Collaborative research efforts from many research groups to improve stress tolerant crop plants are awaited and we hope in the future the results of these studies will contribute to the sustainable food production in developing countries.

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