

Review

Plant signaling in stress

G-protein coupled receptors, heterotrimeric G-proteins and signal coupling via phospholipases

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Plant growth and development are coordinately controlled by several internal factors and environmental signals. To sense these environmental signals, the higher plants have evolved a complex signaling network, which may also cross talk with each other. Plants can respond to the signals as individual cells and as whole organisms. Various receptors including phytochromes, G-proteins coupled receptors (GPCR), kinase and hormone receptors play important role in signal transduction but very few have been characterized in plant system. The heterotrimeric G-proteins mediate the coupling of signal transduction from activated GPCR to appropriate downstream effectors and thereby play an important role in signaling. In this review we have focused on some of the recent work on G-proteins and two of the effectors, PLC and PLD, which have been shown to interact with $G\alpha$ subunit and also discussed their role in abiotic stress tolerance.

Introduction

Plants perceive a wide range of external and internal signals, which are used to regulate various responses in development and adaptation.¹ The exogenous signals include light, temperature, mechanical perturbations like wind, humidity, CO_2 , edaphic factors like soil water and nutrients, and gravity while the endogenous signals include growth regulators, developmental regulators, and metabolites.² Exposure to primary stimuli including light, hormones and pathogen derived elicitors can cause membrane depolarizations of the plant cells within 10-30 seconds, which is linked to early Ca^{2+} influx and anion efflux through ion channels.³ Being themselves static, plants have to have very efficient systems to respond to the environment that fluctuates throughout the day. It seems that the signals are mostly perceived at the level of membrane and therefore transmembrane events are the likely routes for signal generation and transduction. In plants, the best-characterized plasma membrane-based receptors are of two kinds: (i) transmembrane receptor enzymes (usually kinase), (ii) G-protein-coupled receptors (GPCRs). Over the last few years, a number of receptors have been identified in plants, which has helped

in understanding the signal transduction network in plants. Presently in plants, the G-proteins are reported to be involved in processes such as ion channel and abscisic acid signaling⁴ and modulation of cell proliferation^{4,5} in Arabidopsis. However, a wide range of processes-including seed germination, shoot and root growth, and stomatal regulation are altered in Arabidopsis and rice plants with mutations in G-protein components. Their role has been also shown in GA-pathways and in some developmental responses. Recently, role of $G\alpha$ in pathogenicity⁶ and biotic stresses^{7,8} has also been indicated. In this review we have dealt with signal coupling through GPCR, G-proteins and phospholipases especially on their role under abiotic stress environmental conditions.

G-Proteins

By late 1970's through the work of Ross, Gilman and Rodbell in animal system, it was known that the production of cAMP by the action of epinephrine was mediated by GTP binding proteins called G-proteins. In the decade of 80's such proteins were purified, characterized and the genes encoding these proteins were cloned. There are two kinds of G-proteins, monomeric and heterotrimeric.⁹ In this article we will briefly comment on the latter class, which as mediators are involved in the transmission of external signals via receptor molecules to effector molecule. The name of heterotrimeric G-proteins comes from the fact that they form a complex of three different proteins: G-alpha ($G\alpha$), G-beta ($G\beta$) and G-gamma ($G\gamma$).^{10,11} The $G\alpha$ in its inactive state is bound to GDP. The $G\alpha$ has molecular mass of 35-46 kDa and is post-translationally modified by myristoylation/palmitoylation, which enhances its association with membranes. Structurally, $G\alpha$ is highly diverse and contains five conserved domains, G_1 to G_5 , which are involved in GTP binding and hydrolysis. In plant $G\alpha$, the p-loop for NTP binding, the DxxGQ motif for GTP hydrolysis and the NKxD motif for guanine recognition are conserved (see ref. 12). The C-terminal part interacts with seven transmembrane receptor and downstream effectors. The N-terminal part interacts with the $G\beta$ - $G\gamma$ dimer and also contains palmitoylation and myristoylation sites. The molecular mass of $G\beta$ subunit is 35-36 kDa and it belongs to the WD40 family of proteins. Four to 16 copies of WD (tyrosine-aspartic acid pair) repeats are present in a single protein with more or less constant distance of 40 (~40-60) residues, hence called WD40. The WD repeats are involved in protein-protein interaction. The $G\gamma$ subunit is more diverse in structure and has a molecular mass of 6-10 kDa and undergoes isoprenylation at C-terminus region at CAAX motif. Each

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G γ subunit carries a lipid chain at C-terminus, which anchors the G β -G γ dimer and the inactive trimer to the plasma membrane.^{10,11}

On activation, as mentioned above G α exchanges GDP with GTP and following dissociation activates downstream proteins. In fact, G α subunit cycle between an inactive GDP bound and an active GTP-bound form. The activated G α subunit (G α -GTP) leads to the dissociation of G β -G γ dimer from G α . Both these moieties interact with various downstream effector molecules and initiate unique intracellular signaling responses. Termination of the active state is achieved by the inherent GTPase activity of the G α form. After the signal propagation, the GTP of G α -GTP is hydrolyzed to GDP and G α becomes inactive (G α -GDP), which leads to its reassociation with G β -G γ dimer to form the inactive heterotrimeric complex.¹⁰

In plants the G-proteins cascade is studied to some extent in *Arabidopsis* and rice and much of it is still not revealed. The work on plant G-proteins has been recently (reviewed by Temple and Jones (2007) ref. 12). The genomes of diploid angiosperms, such as that of the model species *Arabidopsis thaliana*, contains only single canonical G α gene, *GPA1*,¹³ one G β gene, *AGB1*,¹⁴ and two G γ genes, *AGG1* and *AGG2*.^{15,16} Two G α -subunits (*PGA1* and *PGA2*) are reported from pea.¹⁷ It has been shown using domain mutation analysis that myristoylation or acetylation alone is not the only requirement to direct GPA1 to membranes.¹⁸ Like in animal systems, the two G γ subunits in *Arabidopsis* and also rice G γ have CAAX-box at the C-terminal. One of the rice G γ has been found to have higher molecular weight of around 17 kDa as compared to most of them of around 11 kDa.¹⁹ In mammalian genome G-protein subunits exist as multigene families e.g., alpha has 20, beta 5 and gamma 11 genes. However, in plant genomes so far only one or two genes for each subunit have been found which suggest that G-proteins may be involved in some more restricted, specific signal transduction events.

In plants the G α was found to remain in association with GTP. It was found that the intrinsic GTPase activity was affected by another protein, RGS (Regulator of G-protein Signaling), a 7-transmembrane protein.²⁰ The homologs of this protein have not been reported, though in Medicago data base a plant RGS protein without transmembrane has been reported. Recently, RGS proteins have also been shown to be involved in abscisic acid signaling.²¹

The involvement of G-proteins in various signal transduction pathways was initially implied pharmacologically using various agonists and antagonists. Involvement of G-proteins in light signaling pathways has also been shown. Our own studies had also indicated an involvement of G-proteins in light mediated nitrate reductase gene expression and also in development.^{22,23} A G α cloned from carrot seedlings showed regulation in response to high temperature and high salinity.²⁴ Recently, we have cloned all the three subunits of G-proteins from pea and reported the first direct evidence that G α is involved in salinity and heat stress tolerance while G β is involved in only heat stress tolerance.²⁵

G-Protein-Coupled Receptors (GPCRs)

The GPCR are important as they serve as the gateway for signal transduction induced by ligand binding. In animal system the GPCRs exist as a superfamily of integral membrane protein receptors that contain seven transmembrane α -helical regions, which bind to

a vast variety of ligands and are involved in various signaling pathways.²⁶ Although the human genome contains about 1000 GPCRs but only single gene (*GCR1*) encoding a putative GPCR has been identified in *Arabidopsis thaliana*, which was reported to be cell cycle regulated²⁷ and also involved in ABA signaling in guard cells.²⁸ Using a different bioinformatics tool it was shown that there might be as many as 394 divergent GPCR candidates in *Arabidopsis*.²⁹ Based on sequence homology and functional similarity the animal GPCRs can be grouped into several families such as Family A (rhodopsin-like), Family B (secretin-like), Family C (metabotropic/pheromone), Family D (Fungal pheromone), Family E (cAMP receptors) and Family F (Frizzled/Smoothed).³⁰ Recently, through computational studies of Family A and Family B of GPCRs Vohra et al. (2007)³¹ reported that the plant GCR1 (GPCR) contained homology with Family A, Family B and Family E of GPCRs. The extracellular loops of the GPCR can be glycosylated and contained two highly conserved cysteine residues, which build disulfide bonds to stabilize the receptor structure. GPCR is also known as a guanine nucleotide-exchange factor (GEF) that promotes the exchange of GDP/GTP associated with G α subunit.⁹ The activation of GPCRs though ligand interaction stimulates its GEF activity. The activated GPCRs interact with their cognate G-protein, inducing GDP release with subsequent GTP binding to the G α subunit.^{9,12} The exchange of GDP for GTP resulted in activation of the G α subunit (G α -GTP), which leads to the dissociation of G β /G γ dimer from G α . Both these moieties interact with various downstream effector molecules and initiate unique intracellular signaling responses. After the signal propagation, the GTP of G α -GTP is hydrolyzed to GDP and G α becomes inactive (G α -GDP), which leads to its reassociation with the G β /G γ dimer to form the inactive heterotrimeric complex G α subunit.^{9,12,30,31}

Earlier, the GCR2 from *Arabidopsis thaliana* was reported to be a plant GPCR for the ABA. The ABA is perceived by several different types of receptors in plant cells. At the cell surface, the ABA signal is proposed to be perceived by GCR2, which mediates ABA responses in seed germination, early seedling development and stomatal movement. GCR2 was also proposed to contain seven-transmembrane (7TM) domains. However, recently, Gao et al. (2007)³² found through genetic characterization that there is no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in *Arabidopsis*. Also by using multiple robust transmembrane prediction systems, GCR2 was predicted not to be a 7TM protein, a structural hallmark of GPCRs.³² These authors also reported that loss-of-function mutations in GCR2-LIKE 1 (GCL1) did not confer ABA insensitivity.³² Another putative receptor is GCR1 for which no ligand has been identified yet.³³ A novel GPCR containing a lipid kinase domain has recently been identified in *Dictyostelium* that regulates cell density sensing.³⁴ Recently, we have reported the isolation of a pea *GPCR* gene and the characterization of the encoded GPCR protein and found that this protein interacted with all the subunits of the G-proteins.²⁵

GPCRs have also been reported to control many cellular processes by regulating phospholipid signaling pathways.³⁵ Usually, in response to the stress stimuli, these receptors help to stimulate the phospholipase C (PLC) activity which hydrolyse the membrane phosphoinositide PIP₂ to DAG and the second messengers IP₃. Many receptors also stimulate phospholipase D (PLD), leading to

the generation of the versatile lipid, phosphatidic acid (PA). Usually, the different PLC and PLD isoforms take differential positions in receptor signaling and are regulated by small GTPases of the Ras, Rho and ARF families. The PIP_2 also has signaling capacity by itself and can affect the activity and subcellular localization of PLD and several other proteins. Recently, Oude Weernink et al. (2007)³⁵ presented an overview of how these signaling pathways are governed by GPCRs.

Signal Coupling

Receptor mediated signal transduction in animal systems is either carried forward via the mechanism of protein-protein interaction or via activation of other proteins whose activity leads to the generation of second messengers.^{2,36,37} For example in animal systems, the receptor tyrosine kinase signaling leads to the phosphorylation of the intracellular domain of the receptor at the tyrosine residues to which a specific class of proteins having SH2, SH3 domains bind which in turn bind other proteins like Ras (a small G-protein) that carries forward the signaling via activation of other kinases. Alternatively, other proteins like JAK (janus kinase) bind to the phosphorylated intracellular domain of receptor kinase and in turn phosphorylate another protein, STAT (signal transducer and activator of transcription). The phosphorylated STAT dimerizes and enters the nucleus to activate transcription. Such a scenario is known to operate for serine/threonine receptor kinases. However, as we mentioned earlier this mode of signal operation has not been reported and well studied in plants. The alternative mode is to activate ionic channels in the membrane, for example to regulate calcium uptake and release, or to activate enzymes like phospholipase C or D which through their enzymatic actions can release second messengers or activate heterotrimeric G-proteins, which can effect the activity of enzymes like adenylyclase for the production of cAMP. In plants, in majority of cases, this alternate mode of action, in addition to 2-component signaling and the movement of receptors directly in the nucleus, seems to be operative.³⁸ In this section a brief account of the nature and role of G-proteins, PLD and PLC in stress tolerance are presented.

Phospholipases as G-Protein Effectors

It has been shown that G-protein coupling could lead to the release of second messengers via effectors, which are either ion channels or enzymes. In plants, the presence of the well-known effector molecules of animal systems like adenylyl cyclase, PLC β , GPCR kinases etc have not been established. There is however some evidence that G-protein coupling may be mediated via their interaction with phospholipases. We present below a brief account of the information available on PLA₂, PLC and PLD in plant systems as putative candidates for G-protein effectors.

The lipid derived second messengers are generated by the hydrolysis of phospholipids by the action of specific phospholipases, these are designated as A, C or D (PLA, PLC, PLD) depending on the site of cleavage on the phospholipid backbone. They play an important role in plant growth and development including embryo maturation, seed germination, auxin-stimulated cell division and organ senescence, and also in response to environmental stress including abiotic and biotic stresses.³⁹

In addition PLB is present which removes two fatty acids from phospholipids and thus has both PLA and lysoPLA activities. A few reports do exist on the presence of PLB in plants but there are also reports of several proteins that possess PLB like activities, such as the purified PLA₂ from broad bean⁴⁰ and ricin chain from castor bean.⁴¹ The catalytic properties of these phospholipases are often controlled by their interaction with other proteins like G-proteins, by phosphorylation, interaction with lipids and also activation by calcium.⁴² Recently, the role of sphingolipids in plants has also been suggested. However its role, other than in ABA responses need to be further investigated.⁵¹

PLC. The hydrolysis of phosphoinositide bisphosphate (PIP_2) by specific PLC is one of the earliest key events by which more than 100 extracellular signaling molecules are known to regulate functions of their target cells in animal systems.³⁹ The products of the PLC reaction are the two second intracellular messengers, IP_3 and DAG. The IP_3 induces the release of Ca^{2+} from internal stores and DAG activates protein kinase C. PLCs have been classified into three subfamilies, designated γ (gamma), β (beta) and δ (delta) on the basis of size and amino acid sequences.³⁹ Members of the beta subfamily are activated by heterotrimeric G-proteins and members of the gamma subfamily are activated by protein tyrosine kinase-linked receptors. The mechanism of activation of the delta subfamily members still not well known. Despite differences in their regulation, PLCs of all three subfamilies have similar catalytic properties. They are specific for phosphoinositides, hydrolysing PI, PIP, and PIP_2 but not the 3-phosphate-containing phosphoinositides.^{43,44} They are dependent on Ca^{2+} for activity and their substrate specificities are controlled by Ca^{2+} levels.

There are different families of PLC in animal system; PLC beta, PLC gamma and PLC delta. All of these have pleckstrin homology (PH) domain, EF hand domain, X and Y catalytic domain and C2 domain (protein kinase C-conserved 2 domain). In PLC gamma there are SH2 and SH3 domains between X and Y region. The beta form has an extended C-terminal region. The delta is the shortest form. In plants the first cDNA clone encoding delta phosphoinositol-specific PLC (PI-PLC) was isolated from soybean and was localized in cytoplasm and plasma membranes.⁴⁵ It was found that there are different isoforms in plants and three isoforms of PI-PLC were cloned from potato leaves.⁴⁶ The isolation and characterization of PI-PLC from tobacco and pea was done and shown to be regulated by light in a tissue specific manner.⁴⁷ Cloning of (PI-PLC) cDNA from a number of plant species revealed that they have only the delta form of PLC. When compared, to other PLC's plant PLCs show both X and Y domains, and the C2 domain, however PH domain is absent in the plant PI-PLC delta (Fig. 1A). The X and the Y domain are the catalytic regions, which are separated by variable length of amino acids (in different organisms), however they come together in a 3-D structure. The C2 domain in plant PLC delta was also shown to bind calcium.⁴⁷ Recently, Echevarría-Machado et al (2007)⁴⁸ reported a partial purification of membrane-associated PLC. They found that there are at least two forms (57 and 67 kDa in sizes) of membrane associated PLC in transformed roots of *Catharanthus roseus*, which are differentially regulated during transformed root growth.⁴⁸

The involvement of PLC in stress signaling has been indicated in a number of studies. The genes encoding PI-PLC were found to be induced to a significant extent under environmental stresses. A role

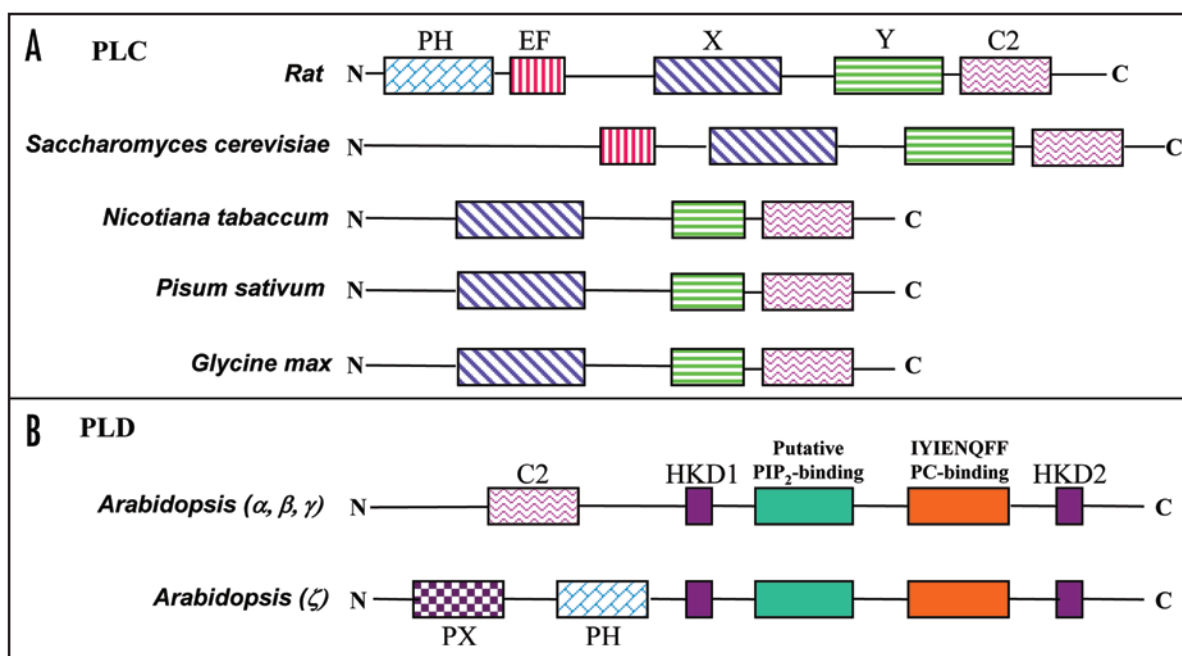


Figure 1. Domain organization of different PI-specific PLC and PLD. (A) Comparison of the domain structures of plant PLCs with the protein from other sources. It shows that plant PLC delta (the only form reported in plants) does not have PH and EF hand domains. (B) The structures of Arabidopsis PLD (α , β , γ and ζ s).

of PI-PLC in the generation of ABA-induced oscillations in $[Ca^{2+}]_{cyt}$ has been suggested. PI-PLC also plays an important role in the gibberellin-induced expression of α -amylase molecules closely related to the germination processes of rice and in secondary ABA responses. Liu et al., (2006a and b)^{49,50} reported that PIP₂-PLC plays a key role in free salicylic acid and ABA-associated thermotolerance resulting from heat acclimation. Since calcium acts as a molecular switch to trigger downstream signaling events and the proline accumulation occurs in various plants in response to environmental stresses (especially drought), hence the calcium signaling via PI-PLC was reported to be essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in *Arabidopsis*.⁵¹ These results also demonstrated the specific involvement of lipid signaling pathway to discriminate between ionic and nonionic stresses.

Role of PLC in Avr-induced disease resistance has also been implicated. In this case PLC/diacylglycerol kinase (PLC/DGK) mediated production of PA was found to be involved in disease resistance signaling.⁵² Recently, Chen et al (2007)⁵³ have shown that the PLC/diacylglycerol kinase (PLC/DGK)-mediated signalling plays an important role in benzothiadiazole-induced oxidative burst, hypersensitive response, and activation of defence response in rice. The involvement of PLC/DAG in PA formation was also shown under aluminium stress in *Coffea arabica* suspension cells.⁵⁴ Dowd et al. (2006)⁵⁵ and Helling et al (2006)⁵⁶ reported that *Petunia* phospholipase C1 is also involved in pollen tube growth.

PLD. PLD catalyzes the hydrolysis of phospholipids, at their terminal phosphodiester bond to phosphatidic acid (PA) and a free polar head group such as choline or inositol. The activation of PLD in the cell generates signaling messengers and is involved in a wide range of cellular processes, including stress and defense response, meiosis, phytochrome action, membrane metabolism and vesicular trafficking.^{4,57} The activity of PLD is highly regulated and its cellular

regulation is often coordinated with the networks of other cellular signaling machinery.

The PA is a second messenger molecule, which is involved in many fundamental cellular processes. PLD and its product PA both are involved in a number of signalling pathways regulating cell proliferation, membrane vesicle trafficking and defence responses in eukaryotic cells. Potocký et al. (2003)⁵⁸ had reported that PLD and PA have also involved in the process of polarised plant cell expansion as represented by pollen tube growth.

Animal PLD activity is known to be stimulated by a large number of cell surface receptors and is elaborately regulated by intracellular factors, including protein kinase C isoforms, small GTPases of the ARF, Rho and Ras families and, particularly, by the phosphoinositide, PIP₂. The PIP₂ acts as substrate for the generation of second messengers by PLC and also recruit and/or activate a variety of actin regulatory proteins, ion channels and other signaling proteins, including PLD, by direct interaction. The synthesis of PIP₂ by phosphoinositide 5-kinase (PIP5K) isoforms is known to be regulated by small GTPases and PA. Recently, Oude Weernink et al (2007)⁵⁹ have described the regulation of PLD by membrane receptors and also suggested that the close encounter of PLD and PIP5K isoforms with small GTPases permits the execution of specific cellular functions. PLD has been identified and purified from many plants.

In higher plants, the PLD encoding genes constitute a large gene family. The cDNA for PLD has been isolated from many plant species, such as castor bean, *Arabidopsis*, cabbage, tobacco, rice and maize.³⁹ There are at least 12 members of the PLD family in *Arabidopsis thaliana*^{15,60} and 17 PLD members found in different chromosomes have been identified in rice.⁵⁷ All PLDs cloned from plants require Ca²⁺ for activity. Each of the C2-PLDs is inferred to have a Ca²⁺/phospho-lipid-binding C2 domain of ~130 amino acid residues near its N-terminal. Two animal-like Arabidopsis PLDs

(PLD ζ 1 and ζ 2) do not have C2 domain, but are equipped with Phox homology (PX) domain (Fig. 1B).

The PLD in plants have been shown to have different domains (Fig. 1B). The most conserved domain is the HKD motif (HxKxxxxD/E) that is present two times. They are away from each other and form the active site. Between these motifs are the PIP₂-binding site and a PC-binding site (IYIENQFF motif). The N-terminus has the C2 domain for calcium binding which is unique to plant PLDs. In some PLD (PLD ζ), one finds the presence of PX (conserved pro-rich motif) and also PH domain (Fig. 1B) (see ref. 61). The PA generated by PLD can function as a second messenger or can also be further metabolized by phosphatidate phosphatase (PP) to form DAG, which is an activator of PKC in signal transduction pathways. Though there are some reports on the presence of PKC type activity in plants, the genes encoding this and its role in signaling has not yet been established.⁶² It seems therefore that PA may be playing a major role for signal transduction in plant systems. Role of PLD has been studied under different stress conditions (see refs. 63-65). It was found that under phosphate limitation stress presence of some forms of PLD were essential for root growth.⁶⁶ The activation of PLD in wounding of leaves and also a key participation in organ senescence-associated cellular changes in higher plants has been shown.⁴ Transgenic studies have shown that the suppressed expression of rice PLD β 1 results in reduced sensitivity to exogenous ABA during seed germination.⁵⁷

By studying its expression under drought stress and also comparing the transcript of this gene in sensitive and tolerant cultivars, it was suggested that PLD may be involved in drought sensitivity and tolerance responses.⁶⁷ Rajashekar et al (2006)⁶⁸ have reported that freezing tolerance in *Arabidopsis* can be induced by suppression of PLD α 1. Recently, grape berry PLD has been cloned and found to be involved in the heat response in post-harvest grape berry.⁶⁹ Early PLD α -dependent transcripts were identified in *Arabidopsis* that were exposed to water stress which further emphasized role of PLD in drought stress conditions.⁷⁰

PLDzeta2 from *Arabidopsis thaliana* has been isolated and reported that it regulates vesicle trafficking and is required for auxin response.⁷¹ Devaiah et al. (2007)⁷² reported that seed quality and viability is enhanced by suppressing PLD α 1 in *Arabidopsis*, suggesting its role in seed deterioration and aging. Therefore, a high level of PLD α 1 is detrimental to seed quality, and attenuation of PLD α 1 expression has the potential to improve oil stability, seed quality and seed longevity.

Interactions Between G-Proteins, PLD and PLC

The mechanism by which G-proteins pass on the message to downstream elements is not very clear in plant systems. Attempts have been made to identify proteins that are coupled to heterotrimeric G-proteins. In a review by Assmann (2002)⁷³ it was mentioned that phospholipases might be regulated by G-proteins. Although from the study on *Brassica napus* it was inferred that there is no direct interaction of PLD α with G-protein in *in vitro* conditions,⁷⁴ yet at present one of the well characterized effector of G α is PLD. It was suggested long back that G-protein activation also stimulated PLD signaling.⁷⁵ It was shown that activation of PLD by ABA was dependent on GTP and inhibitor (like pertussis toxin), that effect G-proteins, blocked PLD activation. These results indicated a role of G-proteins in stimulation of PLD activity.⁷⁶ In fact, Lein and

Saalbach (2001)⁷⁷ provided the first indication for a direct regulation of PLD α 1 by a heterotrimeric G-protein alpha-subunit in tobacco plant. In one of the detailed studies by Zhao and Wang (2004)⁷⁸ it was found that PLD α 1 in fact interacts with GTP α subunit. Using mutated protein and immunoprecipitation techniques, it was found that DRY motif on PLD α 1 was the site of interaction and it preferred GDP state of G α . In fact the GTP state was found to be inhibitory for interaction. It was also found that PLD α 1 interaction with G α stimulates intrinsic GTPase activity of G α (see refs. 64). The physiological significance of this interaction has been recently indicated in stress conditions. It is well known that under drought stress plants tend to accumulate ABA, which controls water loss by regulating stomata opening and closing. Under ABA stress, PA and PLD α 1 seem to interact with G α subunit to mediate ABA inhibition of stomatal opening.⁷⁹

In an earlier study it was found that regulation of DNA synthesis was mediated by the activation of GPCR, leading to G α activation, which in turn affects PLC activity⁸⁰ however, in this study no direct interaction was shown. Recently, we have reported that pea G α and PLC δ proteins interact with each others, moreover, this interaction was further mapped to the carboxy-terminal domain (C2 domain) of pea PLC δ .²⁵ However, in the animal system, the interaction between G α q and PLC β has been reported and has been mapped to its carboxyl terminus.⁸¹ We also found that pea PLC δ protein stimulates the GTPase activity of pea G α . This clearly suggested that PLC δ is one of the effector molecules of the G α subunit. In the animal system, it has been reported that PLC β has the ability to activate intrinsic GTPase activity of G α q. In plant, only the PLC δ isoform has been cloned, which stimulates G α GTPase activity and thereby suggests a cross-talk between them. Whether this interaction has any effect on the activity of PLC still needs to be investigated.²⁵

Besides signaling being transduced via G α , our studies with development of transgenic plants overexpressing the G α or G β subunits and their differential response to salinity and heat stress indicated that G α mediated pathway is responsible for conferring salinity and high temperature stress whereas the pathways triggered by G β lead to heat tolerance.²⁵ On the basis of these results, we propose a model (Fig. 2) depicting the role of G proteins in providing abiotic stress tolerance. Abiotic stress generates signals that are perceived by either GPCR or osmotic sensors present in the cell membrane and this leads to activation of G protein (i.e., dissociation of G α and G $\beta\gamma$ dimer) and hence regulating downstream effectors. It is possible that G α mediated signaling involves activation/ modulation of some downstream effectors conferring salinity and heat tolerance.²⁵ One of the effectors molecules is PLC, and this pathway may lead to an increase in calcium in addition to activation of other pathways (Fig. 2). The burst of calcium increase can lead to the activation of downstream calcium-dependent pathways. In fact the role of calcium in salinity stress tolerance has been elucidated via a number of mechanisms. It is possible that PLC could be involved in salinity tolerance. Thus it seems that cross talk between G α and PLC can be an important step in transducing signals leading to salinity tolerance and also in regulating G α mediated pathways. The PLC can turn the signal off by activating the GTPase activity of G α . The other possibility could be the production of PA via PLC-DAGK or via PLD which in turn can activate downstream components leading to stress tolerance as has been shown in other systems. Overall, the discovery of the cross-talk

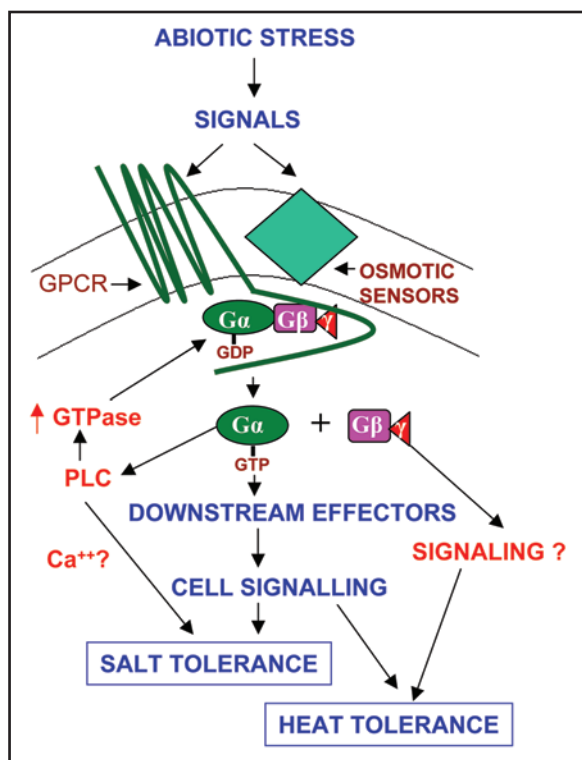


Figure 2. Schematic representation of possible role of heterotrimeric G-proteins in providing salinity tolerance to plants. On basis of interactions of $G\alpha$ and PLC and the role of $G\alpha$ in salinity stress tolerance, the possible mechanism of salinity tolerance may operate through PLC, whereas, mechanism of heat tolerance still remains to discover.

between $G\alpha$ with PLC and PLD should make an important study for better understanding of G-proteins/PLC/PLD-mediated stress signaling pathways in plants.

In addition to interaction with PLC and PLD, there are reports that G-proteins may interact with other proteins also. Using yeast-two-hybrid analysis, the Arabidopsis $G\alpha$ was found to interact with cupin-domain protein designated pirin (PRN1) in both GDP and GTP state.⁸² A proteomic profile of rice dwarf mutant, which lack $G\alpha$ subunit, revealed down regulation of seven seed embryo proteins. One of these, receptor for activated C kinase (RACK) also increased in lines where $G\alpha$ was constitutively overexpressed. These studies showed that RACK is regulated by $G\alpha$.⁸³ One of the protein involved in blue light mediated synthesis of phenylpyruvate and phenylalanine, prephenate dehydratase protein (PD1) was found to have strong interaction with GPA1 of Arabidopsis. The activated GPA1 by GPCR was found to activate PD1 thus suggesting involvement of these three components in signal transduction in light regulation of phenylalanine and subsequent metabolites derived from it.⁸⁴

Cross Talk of Phospholipases with Reactive Oxygen Species (ROS)

Though not directly related to activation via G-proteins, it has been shown that cold induced activation of PLC and PLD leads to expression of different genes. This was shown by blocking PLC/PLD activation by inhibitors like U73112 and ethanol, respectively.⁸⁵ In many cases it has been shown that in response to stress

overproduction of ROS commences. These are detoxified by antioxidants and also by antioxidant enzymes. Hence there may be a relationship of ROS signaling to lipid signaling. It was shown earlier that depletion of PLD α in Arabidopsis decreased PA production as expected but there was also decrease in superoxide production. Addition of PA enhanced synthesis of superoxide, which suggested an important role of PLD in superoxide generation.⁸⁶ Infact it has been suggested that the mechanism by which PLD δ positively contributes to stress tolerance is through signaling mechanisms that induce resistance to damage that is caused by ROS.^{87,88} It seems that different PLDs function differently with respect to ROS signaling. PLD δ is activated by H₂O₂ whereas PLD α 1 and PA are involved in the production of ROS (see ref. 64 and references therein). Recently, salt stress mediated increase in ROS was found to be mediated via phospholipid-regulated signaling pathways.⁸⁹ In this role of PI-kinases was shown but there was no mention for the involvement of PLC.

One of the second messenger related to stress responses is NO. It was found that NO is required for the production of lipid second messenger, PA, via activation of PLC and DAG in tomato cell cultures treated with xylanase, a fungal elicitor. PA was required for xylanase induced ROS production.⁹⁰ These results indicated that PLC/diacylglycerol kinase-derived PA represents a novel downstream component of NO signaling cascade during plant defense. This shows cross talk between two different signaling pathways during plant defense.

Conclusions and Perspectives

Plants are constantly exposed with many biotic and abiotic environmental stresses, which cause considerable losses in crop yields worldwide, while the demand for food and energy is on the rise.^{91,92} Recently, Walley et al. (2007)⁹³ beautifully described that mechanical wounding stress also induces both the biotic and abiotic stress responses via a novel cis-element. The mechanisms by which plant cells perceive the signals and transduce these into the cells are the central focus of plant biologists. In general, the plant do not follow exact same signaling cascades described in nonplant systems, but contain several unique components and unique unexpected cross-talk among signaling components. With the publication of the complete sequence of *Arabidopsis* genome and its analysis, it seems that plants encode a very large number of proteins that may be involved in signal processing. This may be because of the fact that plants are sessile and therefore have to very effectively perceive the environmental variables for their growth, development and adaptation. And also since their survival strategy is of a defensive nature and not an offensive one. To meet these demands they have on one hand retained some of the components of bacterial signal pathway, as for example the two-component receptors for hormones, and on the other hand have some components of higher eukaryotic animal systems.

Since $G\alpha$, PLC and PLD are involved in stress tolerance, it seems that cross talk between these can be an important step in transducing signals leading to stress tolerance and also in regulating $G\alpha$ mediated pathways. The downstream elements of this pathway that are finally regulating gene expression leading to the production of specific metabolites required for tolerance have not been elucidated. Also the mechanism by which the $G\alpha$ signaling is turned off is not clear. Our

preliminary work suggests that the PLC can turn the signal off by activating the GTPase activity of $G\alpha$ thereby regulating the function of G-protein. The recent results on the role of $G\beta$ in stress tolerance need further work to find out its downstream interacting partners. The outcome of this research will lead to a better understanding of stress signaling pathways and will also be useful for its application in agricultural biotechnology.

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