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Heterotrimeric G Proteins: Key Players in Plant Growth and Stress Responses

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ABSTRACT

Heterotrimeric G proteins are large membrane-associated G proteins with subunits (Gα), (Gβ), and (Gγ) present in the plasma membrane in eukaryotes. They are signal transducers that play an important role in signaling pathways in various living organisms. In the inactive stage, Gα contains guanosine diphosphate and forms the heterotrimeric complex with G$\beta\gamma$ dimer which is activated by nucleotide exchange on Gα for guanosine triphosphate (GTP), GTP bound Gα dissociates from G$\beta\gamma$ dimer and modulates the effector for downstream signaling. Regulator of G protein signaling (RGS) protein is a regulatory point of G protein activation and stimulates rate-limiting GTPase activity of Gα subunit. G-protein research in plants is getting a lot of attention recently due to the emerging roles in modulating abiotic and biotic stress responses. An increase in the frequency of extreme weather and climate change has accelerated the adverse effect from plant stresses which are detrimental for plant growth. G-proteins plays important role in various functions that enhances plant growth and development, regulation of hormones, signals, stresses and plant defense responses. Furthermore, future research should be directed towards the discovery of novel components and their signaling mechanisms.

Highlights

⊙ Guanine nucleotide binding proteins (G- proteins) are cytosolic proteins that are present in trimeric complex with α, β & γ subunits in plants.

⊙ They act as signal transducers that regulate multiple signaling pathways in eukaryotes.

⊙ Heterotrimeric G- proteins play vital role in plant growth and development in many plant species.

⊙ Besides roles in plant growth, they are also important for mitigation of abiotic (salt, drought, ozone, temperature) stresses and plant defense responses by inducing different mechanisms.

Keywords: Abiotic stress, G proteins, RGS protein, GTPase, Biotic stress.

The potential climatic abnormalities and changing environmental conditions enable plants to experience numerous abiotic and biotic stresses during their growth and development. Abiotic stresses are drought, elevated temperature, saline conditions, cold, high ultraviolet (UV) radiation, nutrient stress and accumulation of heavy metals in the soil, etc which have been shown to be detrimental for crop production at different developmental stages, also influencing the occurrence of biotic stresses like spread of pathogen, insects-pests, weeds, etc and prove to be potential threat in the future. They not only influences plant morphological responses but also affect crop defense system as well (Scherm and Coakley 2003). Plants, though, have developed several mechanisms to cope with these environmental abnormalities. Despite morphological adaptations, they have also intricated several molecular pathways and mechanisms to deal with these stress responses which include signal perception by receptors, signaling via molecules

and proteins, gene expression (Pandey *et al*. 2015). Extensive study in the areas of stress tolerance mechanisms has come across in integrating the potential functions of G-proteins in stress biology. This review aims at unraveling the different fundamental roles of G-protein science in controlling plant morphological responses and different adaptations towards stress responses. Heterotrimeric G proteins refer to as guanine nucleotide binding proteins present at plasma membrane of eukaryotic cells. These are cytosolic protein categorized as large membrane associated G proteins consisting of 3 subunits namely Gα, Gβ and Gγ forming a heterotrimeric complex. In early 1970s, G proteins were recognized as a signal transducer which links hormone receptor and adenylyl cyclase in mammals (Urano *et al*. 2013). The Ross, Gilman and Rodbell in late 1970s showed that GTP binding proteins (G- proteins) are involved in production of cAMP by action of epinephrine (Tuteja *et al*. 2008). The first α- subunit of G protein in plants was cloned from *Arabidopsis* (AtGPA1) in 1990 (Urano *et al*. 2013). Later in 2007, the self replicating property of G protein was discovered.

Plant's genome contains different group of G proteins unlike animals. A typical angiospermic plant contains two kinds of Gα viz., a canonical Gα and non-canonical extra-large Gα, one type of Gβ and three forms of Gγ i.e., Type A, Type B and Type C Gγ subunits (Wu *et al*. 2018). Human genome encompasses 23 Gα, 5 Gβ and 12 Gγ subunits whereas the *Arabidopsis* genome comprises of one canonical Gα (GPA1), 3 non-canonical Gα like extra-large G protein (XLG1, XLG2 and XLG3), one Gβ (AGB1) and 3 Gγ subunits i.e., AGG1, AGG2 and AGG3. G- proteins have been extensively studied in several crop species like dicots (*Arabidopsis thaliana*, soybean, pea) monocots (maize, rice), gymnosperm (*Pinus taeda*) and in bryophytes (*Physcomitrella patens*) which possess two copies of Gβ and Gγ genes but no canonical Gα. The rice genome, on the other hand, consists of one Gα (RGA1), Gβ (RGB1) and 3 Gγ (RGG1, RGG2 and RGG3) subunits, respectively.

Functions of Heterotrimeric G- proteins in Plants

Several researches over past few decades have reported that heterotrimeric G-proteins play important functions in various functions that enhance plant growth and development, regulation of hormones, signals, stresses, etc. In humans, they are basically an important signal transducing agents in the cells. They are involved in deregulation of GPCR activity and downstream circuits were reported in many diseases like schizophrenia, Alzheimer, cancer, obesity, hypertension and diabetes (Graneir 2012; Hayre 2013; Thathiah, 2011). GPCR signaling is major area of cell biology and therapeutics and its functioning has been most widely studied in protein function (Kamato 2015). In animals, they also transmit extracellular signals like hormones, neurotransmitters, light, taste, etc into intracellular signaling components. Physiological and genetic analysis reveals the functions of heterotrimeric G proteins in various plant physiological and developmental processes. The study of G-proteins in *Arabidopsis* showed that it regulates several functions like germination of the seed (Ullah *et al*. 2002), root cell division (Chen *et al*. 2006), modulates cell proliferation (Chen *et al*. 2003), regulates nitrate, cell wall, immunity and light responses (Chakraborty *et al*. 2019), modulates responses to plant hormones (Mishra *et al*. 2006; Pandey *et al*. 2004). The heterotrimeric G-proteins have been extensively studied in other plant species like rice, soybean, maize, pea, *Camelina* etc. In rice, heterotrimeric G- proteins regulate nitrogen use efficiency (Sun *et al*. 2014), Gγ proteins (DEP1, GGC2) determines grain size in complex with Gβ subunit (Sun *et al*. 2018). In maize, it controls shoot meristem development, immune responses and enhances agronomic traits (Wu *et al*. 2019; Wu *et al*. 2018), controls cell expansion in *Camelina sativa* (Choudhary *et al*. 2019), regulates ABA and auxin signaling in tomato (Subramaniam *et al*. 2016), nodule formation in soybean (Choudhary *et al*. 2015), elongation of culm and grain size in barley (Wendt *et al*. 2016). XLG protein, a functional and biochemical form of canonical Gα controls the development, sporophyte formation and life cycle completion in *P. patens*. Mudgil *et al*. (2016) reported that G- proteins play vital role in mediating the glucose effect on root system architecture in *Arabidopsis* through auxin patterning and transcriptional control. AGB1 acts as sensor component of glucose or carbon nutrient status in roots and modulates root growth. Ferrero-Serrano *et al*. (2017) studied photoavoidance and photoprotection of a dwarf rice mutant with erect

leaves (d1) where RGA1 gene encoding Gα subunit of G-protein is non-functional. Leaves of d1 has lower leaf and high photochemical reflectance index compared to wild type (WT) indicating increased photoavoidance and more efficient light harvesting. G-proteins have important role in development of shoot apical meristem (SAM) in maize. Thus, most of our knowledge about G- proteins from plants comes from studies which depicts that they control various physiological and developmental processes in plants (Table 1). Although the diverse role of HGPs has been identified in plants, the detail mechanism of their action and signaling pathway are needed to be explored.

Table 1: Physiological functions of Heterotrimeric G- proteins in plant species

G-protein Subunit	Interacting protein	Species	Physiological Functions	References
Gα (GPA1)		*Arabidopsis thaliana*	Regulates seed germination, modulates root cell division	Ullah *et al.* 2002; Chen *et al.* 2006
GPA1	GCR1	*Arabidopsis thaliana*	Regulates nitrate, immunity and light responses.	Chakraborty *et al.* 2019
GPA1	AtPirin1	*Arabidopsis thaliana*	Early seedling development	Lapik *et al.* 2003
GPA1		*Arabidopsis thaliana*	Regulates stress, nitrate andphosphate responses, flavonoid biosynthesis, fruit/seed development	Chakraborty *et al.* 2015
GPA1	THF1	*Arabidopsis thaliana*	Sugar signaling pathway	Huang *et al.* 2006
GPA1	ABA (ABA)- insensitive 1 PLDα1	*Arabidopsis thaliana*	Effect of ABA on stomatal closure and opening.	Mishra *et al.* 2006
Gα (RGA1)		*Arabidopsis thaliana*	Regulates photoprotection and photoavoidance.	Ferrero-Serrano *et al.* 2017
Gα (COMPACT PLANT 2)		Maize	Controls shoot meristem size via CLAVATA signaling	Bommert *et al.* 2013
XLG	Related to vernalization (RTV1)	*Arabidopsis thaliana*	Controls vernalisation and flowering	Heo *et al.* 2012
XLG		*Physcomitrella patens*	Sporophyte formation and completing life peroid	Hackenberg *et al.* 2016
XLG		*Arabidopsis*	Root morphogenesis	Ding *et al.* 2008
Gβ (AGB1)		*Arabidopsis*	Affects leaf, flower and fruit development	Lease *et al.* 2001
GPCR	GCR1	Lotus	Controls nodule formation	Rogato *et al.* 2016
Gβ (CsaGβ)	Patatin-like phospholipaseIII δ (pPLAIIIδ)	*Camelia*	Controls cell and organ shape by affecting lipid metabolic pathway	Roy *et al.* 2019
Gβ(AGB1)	N-MYC down regulated like (NDL)	*Arabidopsis*	Regulate auxin transport and root architecture	Mudgil *et al.* 2009
AGB1	Nonphototropic hypocotyl 3 (NPH3)	*Arabidopsis*	Regulate phototropism	Kansup *et al.* 2013
AGB1	Bri-EMS suppressor 1 (BES1)	*Arabidopsis*	Regulate BR signaling and cell elongation	Zhang *et al.* 2017
Gγ (AGG3)		*Arabidopsis*	Guard cell K^+-channel and morphological development	Chakraborty *et al.* 2011
Gγ (GS3 and DEP1)		Rice	Yield improvement and signaling network	Botella 2012
Gγ (DEP1)		Rice	Efficient nitrogen use efficiency	Sun *et al.* 2014
Gγ (HvDep1)		Barley	Regulates culm elongation and grain size	Wendt *el al.* 2016

Heterotrimeric G protein signaling

The heterotrimeric G proteins complex are located in plasma membrane which acts as molecular switches to regulate multiple signaling pathway in eukaryotes. The core G protein component comprises of Gα, Gβ and Gγ subunits. The classical model of HGPs signaling describes that this G protein complex swaps between active and inactive stages depending on nucleotide bound form of Gα. In inactive state, Gα which is bound to GDP also remains associated with G$\beta\gamma$ dimer. Resumption of G protein signaling is mediated by its activation by ligand-binding thus causes change in conformation of GPCRs. The activated GPCR functions as guanine nucleotide exchange factor (GEF) which catalyzes exchange of GDP to GTP situated on Gα protein resulting in dissociation of Gα subunit with G$\beta\gamma$ dimer. GTP- Gα monomer and G$\beta\gamma$ dimer interacts with downstream effectors state of signaling. Activation and deactivation of G-protein requires synchronization for effective and continuous signaling (Ross *et al.* 2008). Since the inherent rate of GTP hydrolysis by Gα is slower than GDP/GTP exchange rate, the proteins are required that can accelerate GTPase activity of Gα. The GAP proteins thus enhance the rate of GTP hydrolysis by interacting with Gα proteins and facilitate effective deactivation and continuation of cycle (Pandey *et al.* 2018). GPCRs, on the other hand, are important gateway for signal transduction induced by ligand binding. They are seven transmembrane α helices with extracellular N- terminal tail and intracellular loops with C-terminus involved in G protein receptor regulation (Stewart *et al.* 2015). GPCR binding to Gα subunits are specific and allows ligand specific modulation of downstream signaling in cells. The RGS proteins are most well known GAPs of Gα proteins (Stewart *el al.* 2015; Siderovoski *et al.* 2005; Ross *et al.* 2008). The human genome consists of 23 Gα, 5 Gβ, 12 Gγ, 37 RGS and about 1000 GPCRs. Compared to this, the G-proteins are extremely limited in plants i.e., 1 canonical Gα, 1Gβ, 2 canonical Gγ, 1unique RGS and no GEF activity possessing GPCR, even though additional non-canonical plant specific proteins exist and function together with G- protein cycle (Stateczny *et al.* 2016; Urano *et al.* 2014; Colaneri *et al.* 2014). Several studies have been made during past few decades to underline the importance of G- protein signaling

in plants. They play pivotal roles in regulation of multiple plant growth and developmental processes as roles of G protein subunits have been demonstrated in controlling seed germination, seedling development, ion channel regulation, stomatal development. G-proteins have immense role in hormone signaling, sugar sensing, light signaling, ROS mediated signaling in many plant species. Unlike animals, in plants the exchange guanine nucleotide in G alpha is mediated in absence of GPCR and the intrinsic hydrolysis rate is slow. In animals, G proteins bind GDP and removal of this nucleotide allow GTP to bind required a receptor having GEF activity, whereas in plants, G proteins are self- activating, spontaneously release GDP and binds GTP *in vitro* (Urano *et al.* 2014). Plants possess canonical and unique G protein components. Their basic biochemistry is, moreover alike from that of metazoans G- proteins, where the classic GPCRs are required for exchange of GTP from GDP for Gα protein activation which is intriguingly missing from plant genomes. Hence, mechanism of G- protein action in plants is slightly dissimilar from metazoan systems.
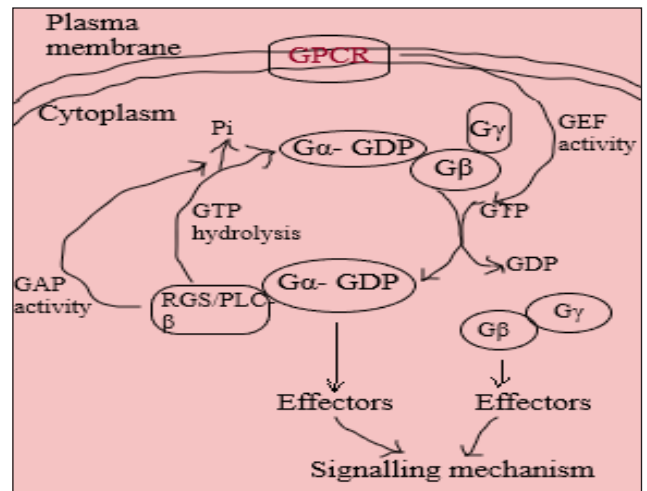

Fig. 1: G protein signaling mechanism (Source: Pandey, 2019).

Heterotrimeric G- protein signaling mechanism

Heterotrimeric G- proteins consist of Gα, Gβ and Gγ subunits. GPCRs and GAPs mediate GDP to GTP exchange reactions of Gα and slow inherent GTPase activity of Gα proteins, respectively. In active state, G proteins interact with downstream effectors to transduce signal.

Activation of G- proteins in plant system

G protein cycle continuum is influenced by many factors which together regulate its rate and continuity. The activation cycle depends on GDP/ GTP exchange, GDP dissociation rate from Gα and rate at which GTP binds on Gα protein, whereas deactivation depends on GTP hydrolysis by Gα & several factors that accelerates the processes (Pandey and Vijaykumar 2018). Thus, both conserved and novel signaling mechanism operate during plant G- protein signaling. In metazoan system, activation of G- proteins occurs due to GEF activity of GPCRs which is missing in plant system. Although they possess several proteins having 7 transmembrane features similar to mammalian GPCR which interacts with Gα proteins in plants. There are several underlying possible mechanisms for the activation of G proteins signaling pathway in plants. Although the canonical, GEF activity possessing GPCRs could possibly be present in plants but has not been identified yet. Many proteins present in plants interact with Gα subunit. GCR1, a well characterized GPCR like *Arabidopsis* protein has significant genetic evidence that it plays fundamental in pathways regulated by *Arabidopsis* Gα protein, GPA1 (Apone *et al*. 2003; Pandey and Asmann 2004; Pandey *et al*. 2016; Warpeha *et al*. 2006; Chakraborty *et al*. 2015a, b). The plant G-proteins are self activating as discussed earlier hence do not require GPCR (Urano *et al*. 2012a, b). This can be hypothesized from a fact that under *in vitro* condition, *Arabidopsis* GPA1 possesses higher rate of GTP binding and displays slow GTPase activity. In case of *in vivo*, the cells contain increased concentration of GTP, a Gα protein will substantially remain in GTP bound conformation. This implies that Gα remains active unless it is deactivated which opposes the existing established paradigm (Pandey *et al*. 2018). One another possibility exists which confer that RLKs are involved in activation of Gα proteins that further interact with G-protein signaling pathway in plants. RLKs have been involved in defense related signaling but involvement of the same during plant development has also been witnessed. RLKs regulate the activity of SAM development in maize. Genetic screening in maize revealed CT2 is an interactor of CLV2 as CLV and WUSCHEL signaling pathways controlled SAM development in plants. Knocking down either CT2 or FEA2 increased SAM size. RLKs also regulate nodule formation in maize. Genetic analysis demonstrated that Gα proteins negatively regulate while Gβγ or RGS protein positively regulates the nodule formation. Nodulation in soybean is stimulated by perception of rhizobial nodulation factor (Nod factor) by Nod factor receptor 1 (NFR1) containing RLKs. Phosphorylation of RGS proteins promote GAP activity and allows nodule formation by maintaining inactive state of Gα protein (Pandey 2019).

Deactivation mechanism of G proteins

G proteins deactivation mechanism is mediated by GTPase activity, GTP bound Gα hydrolysis to bound GTP and thus regenerate GDP in inactive form, although the exact mechanism of activation and deactivation of plant Gα proteins are still to be determined. Since, the GTPase action of Gα proteins in plants is relatively slow, several proteins with GAP activity are required to synchronise activation and deactivation mechanism. RGS protein is well established and recognized GAP in all organisms. In plants, RGS proteins discovery are characterized transmembrane domain linked to RGS domain. RGS protein acts together with Gα protein which are functionally important for effective signaling via G- proteins. Along with RGS, PLDα1 in *Arabidopsis* also accelerates GTP- hydrolysis by Gα. The phenomenon by which phospholipases act as GAPs is well known in mammalian system where PLCβ isoforms act as GAPs and also as effectors of Gα proteins (Pandey *et al*. 2018; Ross 2008; Xu *et al*. 2015; Litosch *et al*. 2013, Ross 2011). Genetic and biochemical analysis confirmed that RGS1 and PLDα1 in *Arabidopsis* accelerates GTPase activity of Gα (Pandey 2017; Roy Choudhary *et al*. 2016; Hong *et al*. 2016; Zhao *et al*. 2013; Pandey, 2016). They interact among themselves and additionally with core G proteins to form higher order protein complexes *in vivo*.

G- proteins are key regulators of abiotic stress responses

Heterotrimeric G proteins regulate various physiological and developmental processes in plants, additionally influencing abiotic stress responses. Plants cope with several abiotic stresses by evolving numerous molecular mechanisms due

to which plant sense stress signal followed by transduction and generation of response (Pandey *et al.* 2016).

Salt Stress

Salinity stress is one of the most important abiotic stresses which results when salts in arid and semi-arid regions gets accumulated over long period. Salt stress causes ionic, osmotic and oxidative stress, not only controlling growth but also influences photosynthetic rate, turgor and enzymatic activity of plants. In *Arabidopsis,* AGB1 efficiently regulates salt tolerance by modulating transcription of genes related to biosynthesis of proline, oxidative stress and responses to ABA as AGB1 mutant (agb1-2) shows decreased survival rates, seed germination rates but increase in MDA concentration and concentration of Na^+/K^+ than WT (wild type) with increase in NaCl concentration (Ma *et al.* 2015). Swain *et al.* (2017) studied the role of rice G- protein γ subunit (RGG1) in salinity tolerance in rice. The signaling events facilitated by RGG1 conferred salinity tolerance by increasing gene expression of antioxidant enzymes. Gene encoding Salt Inducible Zing Finger 1&2 (SZF1& SZF2) is involved in XLG pathway for salt stress response (Liang *et al.* 2017). Liu *et al.* (2019) reported that overexpression of mulberry G- protein MaGβ, MaGY1 & MaGY2 in tobacco increased salt tolerance by modulating ROS detoxification. Under NaCl treatment, the agb1-2 mutant and rgs1-2 mutant of *Arabidopsis* have contrasting phenotype of small and large chlorotic leaves, respectively and this phenotype with loss of function mutations suggest that G proteins promotes growth and attenuates senescence (Colaneri *et al.* 2014). It is well known fact that as salt concentration increases the water potential of soil lowers down which causes osmotic stress and limits uptake of water by plants. The knockout mutant of *Arabidopsis* agb1 shows hypersensitivity to salt and leaf blanching, which accumulates more Na^+ and less K^+ in both roots and shoots in hydroponically grown plants (Yu and Assmann 2015). Increase in salt concentration lowers the water potential of soil which causes osmotic stress and limits uptake of water by plants. Gα null mutations in rice and maize bestowed reduced senescence of leaves, degradation of chlorophyll pigments and cytoplasm electrolyte leakage under salt stress (Urano *et al.* 2014).

Drought stress

Drought stress being multidimensional, affects morpho-physiological, biochemical and molecular traits of plants. It substantially depletes leaf water potential, relative water content (RWC) and transpiration rate with increase in leaf temperature of plant (Farooq *et al.* 2009). The rice d1 mutant possess RGA1 gene and encodes GTP binding α subunit exhibits greater stomatal conductance, endured photosynthesis and increased resistance to drought stress than wild type (WT) (Ferrero-Serrano *et al.* 2016). Genetic analysis showed that HGPs plays a vital role in stomatal opening. In *Arabidopsis*, GPA1 and AGB1 antagonistically regulates stomatal density as overexpression of constitutively active form of GPA^{QL} increased stomatal density and, in contrast, the stomatal density enhanced in null mutant of G- protein β subunit (AGB1) but decreased in transgenic lines overexpressing AGB1 (Zhang *et al.* 2008). Oxidative stress and ROS production increases during drought stress which results in cell damage and death. Over-expression of mulberry β subunit in transgenic tobacco enhanced drought tolerance as transgenic tobacco possess increased proline content, decreased MDA and H_2O_2 content with less superoxide free radical accumulation (Liu *et al.* 2017). In rice, it is suggested that RGB1 (rice β subunit) positively stimulates ABA responses and drought stress tolerance and qPE9 (riceγ subunit) negatively stimulates ABA-mediated drought stress responses (Zhang *et al.* 2015). Heterotrimeric g protein signaling plays an important role in modulating stress responsive genes. The study in *Arabidopsis* genome suggests that overexpression of RGS1 stimulates drought tolerance and ABA biosynthetic enzymes (Chen *et al.* 2006). Phenotypic plasticity is a phenomenon in which one genotype displays different phenotype under different environmental conditions thus allowing plant to reimburse inevitable and inhospitable environment. G proteins function as signaling input in crosstalk between hormonal and environmental conditions and hypothesized as plasticity gene. G protein mutant and WT shows significant difference in plasticities for a number of reproduction related traits. For instance, gpa1 or gcr1 possess enhanced plasticity for inflorescence height or fruit number while agb1 mutant consists of reduced plasticity

for inflorescence height, seed number per fruit and total seed production under drought stress (Nilson and Asmann, 2010). Over-expression of AGG3 in transgenic *Camelina* shows enhanced vegetative and reproductive growth, increased photosynthetic efficiency, increased seed size along with higher oil yield per plant under drought stress (Roy Choudhary *et al.* 2014).

Temperature stress

The drastic change in temperature over recent decades induced high or low temperature. High temperature stress often attributed as rise in temperature past threshold level for a period of time which causes irreversible damage to plant growth and development (Wahid *et al.* 2007) whereas low temperature or cold stress is induced by exposure to reduced temperature usually below 0-15°C. The role of G- proteins in temperature stress are yet to be explored as limited studies have been made under temperature fluctuations. Increased temperature sensing in *Arabidopsis* requires Phytochrome interacting factor 4 (PIF4) - transcription factor and changes in H2A.Z chromatin status (Kumar *et al.* 2012). Heat stress response is mediated by multiple pathway and regulatory network. Heat induced Ca^{2+} and ROS signaling plays a vital role in activation of HSFA1 to increase thermotolerance in plants (Mittler *et al.* 2012). HSFA1 is a master regulator of heat stress responses which initiates the transcription cascade of HSPs and other genes and also induces their activity under different stresses like salt, osmotic and oxidative stress to confer tolerance (Liu *et al.* 2011). Bai *et al.* (2018) reported that over-expression of CsGG3.2 (Gγ subunit) in transgenic plant exhibits chilling tolerance in cucumber with increased expression of CBF genes. Overexpression of KE1 gene (positive regulator of CBF3 gene) enhances chilling tolerance in transgenic *Cucumis sativus L.* Transgenic plant accumulated more soluble sugar and proline and showed increased activity of antioxidant enzyme with less electrolyte leakage than WT under cold stress (Liu *et al.* 2011). Overexpression of *Pisum sativum* Gα and Gβ showed thermo tolerance in transgenic tobacco (T_0 and T_1) plants (Mishra *et al.* 2007). The agg 2 mutant of *Arabidopsis* when grown at higher temperature (29°C) exhibits earlier flowering than WT (Thung *et al.* 2013). Wu *et al.* (2012) reported that

plants exhibiting cold regulated gene CbCOR15b expressed in leaves, stems and roots possess greater cold tolerance than control. The QTL protein COLD1 interacts with RGA1 thereby stimulating GTPase activity to activate Ca^{2+} channels in response to chilling stress in rice (Ma *et al.* 2015b).

Ozone stress

Ozone being a secondary pollutant affects the plants at physiological and molecular level. Ozone enters the plant through stomata and breakdown in apoplast to produce ROS (Ludwikow and Sadowski 2008), thus induces oxidative stress and PCD. Long term exposure to ozone causes chlorosis and necrosis, reduced photosynthesis and decreased yield (Wu *et al.* 2018). Genetic evidence reveals that membrane associated GTP proteins play a crucial role in signal transduction by activation of target proteins and transduce extracellular signals to intracellular receptors (Booker *et al.* 2004).
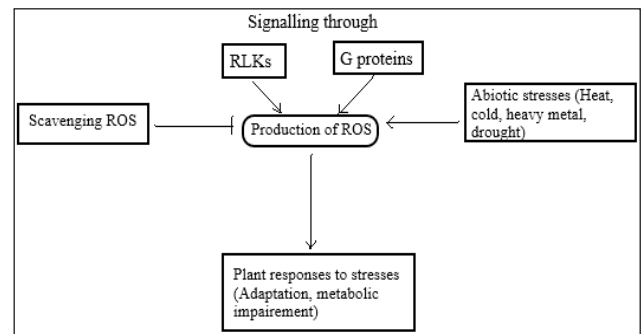

Fig. 2: G protein signaling in abiotic stress
(***Source:*** Lohani *et al.* 2020).

HGPs signaling also controls stress associated physiological responses. *In Arabidopsis*, epinasty was observed in WT instead of gpa1 mutant when treated with ozone (O_3). gpa1 mutant showed decreased leaf chlorophyll content and leaf mass per unit leaf area in response to O_3. ROS production by O_3 is facilitated in early and late times. *Atrboh D and Atrboh F* are cell membrane associated NADPH oxidases which triggers ROS production in initial time while the late ROS signal is described by tissue damage associated component of oxidative burst required only Gα protein. G protein signaling plays an important role in activating intracellular sources of ROS that contribute to first component of triphasic stress mediated oxidative burst in *Arabidopsis* (Joo 2005). Booker *et al.* (2015) studied the potential response mechanism of G-proteins to

Table 2: Function of Heterotrimeric G protein in different abiotic stresses

Plants	Stress	G-protein subunit	Physiological responses	References
Arabidopsis	Salt	Gβ (AGB1)	Modulates gene transcription related to proline biosynthesis, oxidative stress and ABA response	Ma *et al.* 2015
Arabidopsis	Salt	At GPA1, At RGS1	Modulates plant growth	Colaneri *et al.* 2014
Rice	Salt	RGG1	Increased gene expression of antioxidant enzymes	Swain *et al.* 2017
Arabidopsis	Salt	XLG and SZF1, SZF2	NaCl induced stress responses	Liang *et al.* 2017
Tobacco	Salt	MaGβ, MaGγ1&MaGγ2	Modulates ROS detoxification	Liu *et al.* 2019
Rice & Maize	Salt	Gα	Decreased leaf senescence, chlorophyll degradation & electrolyte leakage	Urano *et al.* 2014
Rice	Drought	RGA1	Increased stomatal conductance, sustained photosynthesis	Ferrero-Serrano *et al.* 2016
Tobacco	Drought	AGB1	Higher proline and peroxidase content, low MDA and H_2O_2 accumulation	Liu *et al.* 2017
Arabidopsis	Drought	RGS1	Increased drought tolerance and ABA biosynthetic enzymes	Chen *et al.* 2006
Arabidopsis	Drought	AGB1	Regulation of reproductive trait and plasticity under drought stress	Nilson & Assmann, 2010
Camelina	Drought	AGG3	Increased vegetative and reproductive growth, higher photosynthetic efficiency, higher yield per plant	Choudhary *et al.* 2014
Tobacco	Heat stress	Gα&Gβ	Heat tolerance, higher chlorophyll content in leaf disk of salinity stressed plant	Mishra *et al.* 2007
Cucumber	Chilling stress	CsGG3.2	Increased activity of antioxidant enzymes, decreased ROS production & lipid peroxidation	Bai *et al.* 2017
Rice	Cold stress	COLD1& RGA1	Stimulation of GTPase activity, activation of Ca^{2+} channels	Ma *et al.* 2015b
Cucumis sativus L.	Cold stress	KE1gene	Increased soluble sugar, proline content, antioxidant enzymes, lower electrolyte leakage and MDA content	Liu *et al.* 2010
Arabidopsis	Ozone stress	α&β subunit	Activation of intracellular ROS generating system	Joo *et al.* 2004

O_3 by comparison between *Arabidopsis* null mutants of α and β subunits (gpa 1-4, agb 1-2 and gpa 1-4/agb 1-2) and WT. Transcript levels of GPA1, AGB1 and RGB1 genes were induced by O_3. They further also reported that silencing of α and β G-protein genes resulted in alteration of many processes associated with O_3 injury, including ROS signaling genes, increase in leaf tissue ion leakage with reduced net photosynthesis. Many responses to O_3 stress at physiological level were not necessarily influenced by α and β G-protein (Booker *et al.* 2012).

Role of G proteins in plant defense responses

Heterotrimeric G proteins are particularly involved in plant defense responses. They are involved in various facets of plant disease resistance.

Overexpression of Gα and Gβ subunit results in decreased bacterial multiplication of non-host pathogen *P. syringae pv. tabaci* while overexpression of Gβ and not Gα results in reduced bacterial growth of host pathogen *P. syringae pv. Maculicola* compared to wild type along with altered stomatal aperture by bacterial pathogen in Gα and Gβ mutants, not in single or double mutants (Lee *et al.* 2013). AGG1 and AGG2 proteins have redundant functions in controlling *Arabidopsis* immune response to necrotrophic fungus *P. cucumerina* indicting that susceptibility of agg1-1 agg1-2 double mutant to this pathogen is similar to that of agb1 plant but higher than agg1-1 agg 2-1 single mutants (Delgado- Cerezo *et al.* 2012). In contrast, Trusov *et al.* (2006) reported that AGG1 has specific role in

Table 3: Function of Heterotrimeric G protein in different biotic stresses

Mutant	Species	G-protein Subunit	Biotic stress response
xlg2	*Arabidopsis*	XLG 2&XLG 3	Enhanced susceptibility to *P. synringae* (Zhu *et al.* 2009)
gpa1	*Arabidopsis*	Gα	Reduction in stomatal density (Zhang *et al.* 2008)
agb1	*Arabidopsis*	Gβ	Enhanced susceptibility to pathogen *A. brassicola, B. cinerea, F. oxysporum & P. cucurmerina* (Llorente *et al.* 2005), Reduced elicitor induced ROS production, increased susceptibility to *P.s.t DC 3000 & P. syringae pv. Maculicola* (Torres *et al.* 2013), restricted constitutive cell death & defense responses in bir 1-1, reduced resistance to non-pathogenic bacteria *P.s.t DC 3000 hrcC* (Liu *et al.* 2013).
agg1 agg2	*Arabidopsis*	Gγ	Impaired elicitor induced to *P. syringae* pv. tomato DC3000, reduced MPK4 activation by flg22, suppression of constitutive cell death & defense reponses in bir1-1 (Liu *et al.* 2013), increased susceptibility to P.S.M & *P. synringae* pv. Tabaci (Lee *et al.* 2013), increased susceptibility to necrotrophic fungi *P. cucumerina, F. oxysporum, B. cinerea* (Trusov *et al.* 2006; Trusov *et al.* 2009; Trusov *et al.* 2007).
d1	*Oryza sativa*	Gα	Decreased defense reponse to avirulent strains of *X. oryzae* pv. *oryzae & M. grisea* (Komatsu *et al.* 2004), decreased hypersensitive response to avirulent strains of *M. oryzae* (Suharsono *et al.* 2002).
Silencing of Gα	*Nicotiana benthamiana*	Gα	Impaired elicitor, activated stomatal closure, decreased NO production in guard cells in response to elicitor, attenuation of elicitor induced H_2O_2 accumulation, suppress harpin induced cell death (Zhang *et al.* 2012)
Silencing of Gβ1 & Gβ2		Gβ	

the mediating *Arabidopsis* resistance to necrotrophic fungus *A. brassicola*. Heterotrimeric G-proteins plays an important role in plant defense by mediating ROS production by NADPH oxidases (Vera-Estrella *et al.* 1994). G- proteins influences cell wall components, ROS production and also interacts with JA and MAP kinase signaling network component as plant response to pathogens (Torres *et al.* 2013). Transcriptome analysis revealed that extra- large G protein (XLG 2 and XLG 3) were greatly incited by infection with bacterial pathogen *P. syringae* as loss of function mutation of xlg2 increased susceptibility to the same in *Arabidopsis* (Zhu *et al.* 2009). Studies on the G-proteins provide an evidence that they are involved in resistance to wide range of pathogens. *Arabidopsis* genome consists of 3 non- canonical Gα i.e. XLG1, XLG 2 and XLG 3. The *Arabidopsis* triple mutant xlg1, xlg2, xlg3 and quadruple mutant gpa1xlg1 xlg2 xlg3 showed impaired resistance and no additive effect in susceptibility between triple and quadruple mutant showing that GPA1 does not play role in disease resistance (Urano *et al.* 2016a). In *Arabidopsis*, Gβγ dimer associates with XLG to confer plant immunity as *Arabidopsis* mutant lacking XLG, Gβ and Gγ shows reduced pathogen

defense, development and production of ROS as well (Maruta *et al.* 2015). Study in rice reveals that rice d1 mutant which lacks RGA1 possesses decreased defense responses to avirulent strains of *X. oryzae pv. Oryzae* and *M. grisea* (Komatsu *et al.* 2004). *Arabidopsis* mutant deficient in AGB1 (Gβ subunit) are highly susceptible to pathogens viz., *A. brassicola, B. cinerea, F.oxysporum* and *P. cucumerina* (Llorente *et al.* 2005; Trusov *et al.* 2009), agb1 mutant shows hindered induction of MeJA induced PR genes, PDF12, OPR3 and PAD1 (Trusov *et al.* 2009), whereas SA dependent PR1 expression was enhanced after *P. cucumerina* infection (Llorente *et al.* 2005). Studies on G- protein also reveals that they are actively involved in elicitor induced hypersensitive respone (HR) in plants by VIGS induced silencing of Gα, Gβ and Gβ1 subunit of *N. benthamiana* and G protein complex. The treatment of silenced plants with bacterial and fungal elicitors showed reduced HR which includes stomata closure, NO production and ROS accumulation in guard cells (Pandey *et al.* 2015; Zhang *et al.* 2012). Suharsano *et al.* (2002) reported that rice d1 mutant which lacks Gα gene showed decreased hypersensitive response to avirulent strains of rice blast. Further

analysis also reveals that G protein function in ETI-triggered defense response as there was suppression of ROS production and PBZ1 expression by SE in d1 mutant cell cultures. Heterotrimeric G-proteins are evolutionary novel protein complexes which comprises α, β and γ subunit present in eukaryotes. These mediate PAMP triggered immunity. Flagelline-sensitive 2 (FLS 2), elongation factor TU (EF-Tu) receptor (EFR) and chitin elicitor reception kinase 1 (CERK1) are well known pathogen-associated molecular pattern (PAMP) receptors belongs to RLK family. In *Arabidopsis*, treatment with flagelline derived peptide flg 22, EF-Tu derived peptide elf 18 or chitin trigger the ROS production in WT plants. Mutations in AGB1 leads to reduced ROS production by flg 22 and chitin and complete termination of ROS production by elf 18 which is contrast to GPA1 mutation in which there is no effect on ROS induction by elf 18, flg 22 or chitin. This suggests that AGB1 and AGG1/2 functions downstream of RLKs, FLS2, EFR and CERK 1 to regulate PAMP oxidative burst (Liu *et al.* 2013). RLKs functions parallel to GPCRs in plants and Gβγ subunit which functions downstream to RLKs that converges plant defense signaling upon perception of PAMPs. *Arabidopsis* WT plants possess increased resistance to *P.syringae pv. Tomato(P.s.t) DC 3000* when pretreated with *flg22, elf18* and *chitin* induced resistance was more likely reduced in agb1 and agg1, agg2 mutants in contrast to gpa1 mutant plants (Liu *et al.* 2013). The receptor like cytoplasmic kinase BIK1 also functions downstream to FLS 2, EFR1 and CERK1 that regulates PAMP triggered oxidative burst (Lu *et al.* 2019; Zhang *et al.* 2010).

ABBREVIATIONS

XLG = extra large G- protein, HGPs = Heterotrimeric G- proteins, SAM = Shoot apical meristem, GEF = Guanine nucleotide exchange factor, GPCR= G- protein coupled receptor, RGS = Regulator of G protein signaling, RLKs = Receptor like kinases, NFR1 = Nod factor receptor1, PLDα1 = Phospholipase Dα 1, PLCβ= Phospholipase C, PAMP = Pathogen associated molecular pattern

FUTURE PROSPECTS

Heterotrimeric G proteins are cytosolic proteins which play vital roles in plant growth. Significant researches and studies have been made regarding function of G proteins, RLKs in plants and mammalian system but several questions still remains unanswered. The pathways of regulation of G protein mechanism in monocots and eudicots are still not well understood. The mechanism of action of canonical and novel components of G proteins along with regulatory proteins is needed to be explored. Similarly the mechanism of stress tolerance by heterotrimeric G proteins is not clear. Heterotrimeric G proteins signaling is a complex phenomenon and pathways regulating signaling mechanism, activation, role of protein kinases, phosphatases are further needed to be explored. The functions of G proteins subunit possess only handful of information. The role of Gβγ subunit in defense responses is still needed to be understood. Similarly, only few effectors downstream of subunit Gα and XLG has been recognized in plants system. G proteins have a vital role in nutrient sensing, sugar signaling and controlling plasticity in plants. However, researches have made a significant effort to unravel the mechanism of the same but still more efforts are needed in this direction. Identification of receptors involved in signaling mechanism is one of the major tasks that warrant attention.

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