Title: Role of heterotrimeric G-proteins in improving abiotic stress tolerance of crop plants

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Abstract

As sessile organisms, plants are constantly exposed to a variety of environmental stresses that have detrimental effects on their growth and development, leading to major crop yield losses worldwide. To cope with adverse conditions plants have developed several adaptive mechanisms. A thorough understanding these mechanisms is critical to generate plants for the future. The heterotrimeric G-protein complex, composed of Gα, Gβ, and Gγ subunits, participates in regulation of multiple cellular signaling pathways and have multifaceted roles in regulating stress responses of plants. The complex has two functional entities, the GTP-bound Gα subunit and the Gβγ dimer, both of which by interacting with additional proteins can activate various signaling networks. The involvement of G-proteins has been shown in plants' response to drought, salinity, extreme temperatures, heavy metal, ozone, and UV-B radiation. Due to their versatility and the number of processes modulated by them, G-proteins have emerged as key targets for generating stress tolerant crops. In this review, we provide an overview of the current knowledge of the roles of G proteins in abiotic stress tolerance, with examples from model plant *Arabidopsis thaliana,* where these processes are most widely studiedand from additional agriculturally relevant crops, where their potential is realized for human usage.

1. Introduction

Any divergence from the optimal growth conditions adversely affecting an organism's growth, metabolism and development can be termed as stress (Lichtenthaler, 1998). Plants being sessile are continuously challenged by multiple stresses, both biotic and abiotic (Zhang et al., 2022). The most common abiotic stresses in plants include drought, salinity, extreme temperatures (high/freezing), heavy metal contamination (cadmium, aluminium and arsenate in soil), ozone and UV-B radiation, which individually or in combination affect almost every aspect of their growth and development. The global climate change over the past few decades had compounded the effects of abiotic stresses in plants by significantly affecting crop productivity world-wide, raising major concerns for the future food security (Bita and Gerats, 2013; Jagermeyr et al., 2021).

Plants' response to abiotic stresses can be adaptive, non-adaptive or a combination of both. The non-adaptive responses are usually deleterious due to compromised biomolecule functioning and altered membrane dynamics (Zhang et al., 2022). The adaptive responses, on the other hand, are suitable to the sessile life style of plants. Plants exhibit dramatic developmental plasticity brought about by rapid changes in ion channel activities, gene expressions, chromatin remodelling, post-transcriptional modifications, and translational/post-translational modifications (Bita and Gerats, 2013; Niu and Xiang, 2018; Zhang et al., 2022), which is beneficial when responding to stresses. Studies performed over the years have also emphasized that the adaptive, plastic behaviour of plants is an outcome of the regulation by multiple interconnected genes and their networks (Laitinen and Nikoloski, 2019). Thus, it is pertinent to elucidate the molecular framework of stress responses in plants, with an aim to identify key stress sensors, stress modulators and stress responsive genes. This will eventually lead to breeding stress resilient crops to meet the future food demands.

Heterotrimeric guanine nucleotide binding proteins (G-proteins), comprised of Gα, Gβ and Gγ subunits are key signaling intermediates in eukaryotes (Offermanns, 2003; Pandey, 2019). In metazoan, G-proteins are key mediators of various sensory perceptions, hormones and neurotransmitter signaling and consequently affect almost all aspects of normal growth and development (Neves et al., 2002). Due to their involvement in controlling various human diseases, the signaling mechanisms of G-proteins have been extremely well-characterized in

humans. By recent estimates, G-protein signaling pathways are targets of more than 30% of all pharmaceutical drugs (Li et al., 2020; Yang et al., 2021).

In plants, G-proteins affects every aspect of growth and development and responses to a multitude of exogenous and endogenous cues (Jose and Roy Choudhury, 2020; Pandey, 2019; Tiwari and Bisht, 2022; Wang and Botella, 2022; Zhou et al., 2019). The subunit composition of the heterotrimer and core biochemistry of the Gα, Gβ and Gγ proteins is similar between plants and metazoans. However, several unique components as well as distinct regulatory mechanisms also exist, which are thought to have evolved in response to the unique lifestyle of plants (Chakravorty and Assmann, 2018; Pandey, 2017; Pandey, 2019; Wang et al., 2018; Zhou et al., 2019). Plant G-proteins are involved in modulation of a plethora of physiological processes at the subcellular, cellular, tissue and organ levels. These include control of stomatal movement, regulation of phytohormone signaling (Chakravorty et al., 2011; Hao et al., 2012; Jose and Roy Choudhury, 2020; Lee et al., 2018), response to biotic and abiotic stresses by affecting fundamental processes such as ion channel activities (Chakravorty et al., 2011; Fan et al., 2008), cell division, expansion and differentiation (Chen et al., 2006; Chen et al., 2003; Ueguchi-Tanaka et al., 2000; Ullah et al., 2003; Urano et al., 2015), changes in membrane dynamics and cell wall composition (Chakravorty et al., 2011; Roy Choudhury et al., 2019; Wu et al., 2007; Zhang et al., 2011). In addition, G-proteins are also control key agronomic traits such as water and nitrogen use efficiency and yield by influencing inflorescence and root architecture, seed number and size, and germination potential (Botella, 2012; Cui et al., 2020; Kaur et al., 2018; Liang et al., 2018; Roy Choudhury et al., 2019; Sun et al., 2014; Urano et al., 2015; Vavilova et al., 2017; Wendt et al., 2016; Wu et al., 2018; Zhang et al., 2015).

2. Plant heterotrimeric G-proteins composition

In plants with non-duplicated genomes, the repertoire of heterotrimeric G-proteins is relatively simple compared to the metazoan systems (Pandey, 2019). Plants have two types of Gα subunits, canonical Gα, which shows similarity to other known non-plant Gα proteins and the larger form of Gα, known as extra-large Gα (XLG), in which the Gα domain is fused with an extra, N-terminal region (Chakravorty et al., 2015; Ding et al., 2008; Hackenberg et al., 2016). The Gα protein possesses an intrinsic Ras-like GTPase domain and a unique alpha helical domain with conserved N-terminal myristoylation site at (Gly2), crucial for its membrane anchorage (Figure

1A) (Galbiati et al., 1994). The N terminal portion of XLGs has a cysteine rich region and a nuclear localization signal (NLS) (Chakravorty et al., 2015). The Gα-like domain in XLG proteins has lost some of the residues crucial for nucleotide hydrolysis, but has been shown to bind GTP (Hackenberg et al., 2016; Urano et al., 2016). Recent phylogenetic analysis shows that there are instances of the canonical Gα loss in some plant groups (*e.g.,* Bryopsida mosses) but the XLG proteins are present in all plant groups except Chlorophyceae algae (Mohanasundaram et al., 2022). The Gβ protein harbours N-terminal coiled-coil helices and seven WD40 (Trp and Asp) repeat containing domain, which is implicated in multi-protein complex formation (van Nocker and Ludwig, 2003). Plants have three types of Gγ proteins-types I, II and III (also known as types A, B and C) (Figure 1A) (Roy Choudhury et al., 2011; Trusov et al., 2012). Phylogenetic analysis suggests that the Gγ proteins diverged before the evolution of land plants and underwent considerable changes in their domain structures, resulting in these sub-types (Mohanasundaram et al., 2022). The type-I is the prototypical Gγ subunit with N-terminal coiled-coil domain and C-terminal prenylation motif CAAX ("C" represents cysteine, "A" for any aliphatic amino acids and X denotes any amino acid) involved in post-translational modification and membrane anchorage. The type-II Gγ proteins differs from the type-I Gγ only by the loss of the prenylation motif (Figure 1A), although the proteins are still hypothesized to be localized to the plasma membrane (Botella, 2012; Zeng et al., 2007) . The type-III is a higher plant-specific Gγ protein with its N-terminal region similar to prototypical type I Gγ fused with a highly divergent C-terminal cysteine-rich region (Botella, 2012; Roy Choudhury et al., 2011; Trusov et al., 2012). In Arabidopsis, the G-protein trimeric complex is represented by one canonical Gα, GPA1, three extra-large Gα (XLG1, 2 and 3), one Gβ, AGB1, and three Gγ proteins, AGG1, AGG2 (type I) and AGG3 (type III) (Mason and Botella, 2000; Mason and Botella, 2001; Pandey, 2019). The repertoire of G-proteins in many angiosperms is similar to Arabidopsis; however, plants with recently duplicated genomes have retained most copies of G-proteins. For example, the soybean genome codes for 4Gα, 4 Gβ, 10 XLG and 12 Gγ proteins (Bisht et al., 2011; Roy Choudhury et al., 2011). Similar higher numbers are reported from Camelina, wheat and brassica species (Gawande et al., 2022; Kumar et al., 2014; Roy Choudhury et al., 2014).

3. Plant heterotrimeric G-protein signaling mechanisms

The Gα subunit of the heterotrimer binds guanine nucleotides and switches between GDP-bound, inactive and GTP-bound, active forms. In metazoan, the exchange of GTP for GDP on Gα is facilitated by a plasma membrane-localized, 7-transmembrane containing G-protein coupled receptor (GPCR), which acts as a guanine nucleotide exchange factor (GEF) (Oldham and Hamm, 2008). Upon activation by a GPCR, GTP-bound Gα dissociates from the trimer and releases Gβγ dimer. Both these entities (GTP-Gα and Gβγ) can independently interact with various downstream effectors to relay the G-protein mediated signals (McCudden et al., 2005; Siderovski and Willard, 2005). The inherent GTPase activity of Gα proteins causes the hydrolysis of bound GTP, generating its GDP-bound form, which associates with the Gβγ dimer to reconstitute the inactive trimer. This transition from active to inactive state is also accelerated by the GAP (GTPase-activity accelerating proteins) activity of RGS1 (Regulator of G protein Signaling) proteins (McCudden et al., 2005; Siderovski and Willard, 2005). These core properties i.e., guanine nucleotide-binding dependent trimeric or monomeric Gα proteins, signaling by freed Gα and Gβγ, and regulation of signaling by the GAP activity of the RGS proteins is conserved in plant G-protein signaling as well, but several deviations also exist (Figure 1B). In addition to the unique features of the XLG and type III Gγ proteins, the plant RGS proteins possess a domain containing 7 transmembrane regions, and are plasma membrane localized (Chen et al., 2003; Hackenberg et al., 2017; Mohanasundaram et al., 2022; Roy Choudhury et al., 2012). Classical GPCRs with GDP/GTP exchange activity have not been identified in plants, to date. The only GPCR-like proteins identified in Arabidopsis through a reverse genetic approach, GCR1, has a protein fold similar to non-plant GPCRs and act in G-protein dependent pathways (Pandey and Assmann, 2004), but its GEF activity remains unknown. Instead, the involvement of plasma membrane bound receptor-like kinases (RLKs) in regulating G-protein signaling by phosphorylation/dephosphorylation-based mechanisms appears to be more prevalent in plants (Chakravorty and Assmann, 2018; Liang et al., 2016; Roy Choudhury and Pandey, 2015; Roy Choudhury and Pandey, 2016a; Wang et al., 2018). Similarly, RGS-dependent regulation of G-proteins exists in plants, but many plant groups do not have an RGS homolog in their genome (Bhatnagar and Pandey, 2020; Hackenberg et al., 2017; Mohanasundaram et al., 2022). Various phospholipases may also be involved in the regulation of G-protein signaling (Brandenburg et al., 2014; Jeon et al., 2019; Pandey, 2016; Pandey, 2017; Roy Choudhury and Pandey, 2016b; Roy Choudhury and Pandey, 2017). Furthermore, a

guanine-nucleotide-independent mechanism of regulation has also been proposed (Maruta et al., 2021), implying that the regulation of G-protein signaling mechanisms in plants is flexible, potentially suitable for the sessile nature of the plants and their need to integrate multiple signaling pathways.

4. Roles of G-proteins in regulating abiotic stress responses

G-proteins are known to regulate multiple abiotic stresses in plants. However, most of our current knowledge is based on the results from the model plants *Arabidopsis thaliana*, with some information from crops such as rice, maize, soybean or barley. In the next sections, we will describe the roles of G-proteins in regulation of abiotic stress responses, with the foundational knowledge from Arabidopsis and the extent to which it has been expanded to crop plants.

4.1 Drought stress

Drought stress leads to poor germination, challenges in seedling establishment, wilting of leaves with reduction in leaf number and surface area, reduced plant height, an overall change in root system architecture affecting primary root length, lateral root density and morphology of root hairs, reduced flowering and diminished seed filling, all resulting in significantly reduced yields (Koevoets et al., 2016; Seleiman et al., 2021). Drought stress is primarily sensed by roots and the information is transmitted to the aerial parts of plants via long-distance root–to-shoot signaling, leading to the production of abscisic acid (ABA) in the leaves, a key phytohormone regulating stress response in plants through regulation of stomatal movement and stress responsive molecular changes (Li et al., 2021; McAdam et al., 2016; Schachtman and Goodger, 2008). It is not surprising that several of the ABA-regulated gene expression networks are shared between the roots hair cells and stomatal guard cells (Li et al., 2012).

Regulation of water loss through stomatal pores by modulating guard cell aperture is an important adaptive response of land plants (Buckley, 2019). ABA plays a key role in maintenance of stomatal physiology through regulation of ion fluxes across the guard cell membrane (Assmann and Jegla, 2016; Huang et al., 2019; Kim et al., 2010; Munemasa et al., 2015). ABA regulates the inward potassium and the calcium channels and transporters, influencing the levels of K^+ and Ca^{2+} and consequently of anions such as Cl^- and malate, essentially promoting the closure of open stomata and inhibiting the opening of closed stomata,

in response to water limitation (Assmann and Jegla, 2016; Eisenach and De Angeli, 2017; Kim et al., 2010; Munemasa et al., 2015). This regulation ensures plants' fitness by controlling water loss. Several genetic and biochemical studies have identified G-proteins as direct regulators of ion channel activities and stomatal physiology, implying their direct role in mitigating drought stress (Fan et al., 2008; Jeon et al., 2019; Wang et al., 2001). In Arabidopsis, the availability of knockout mutants of each of the G-protein subunit genes (and their combinations) has allowed for investigating the roles of G-proteins in regulating drought stress using multiple approaches (Fan et al., 2008; Nilson and Assmann, 2010; Pandey and Assmann, 2004). These include elucidating the effects of G-protein function on guard cell ion channel activities, transpiration efficiency, gene expression and proteomic changes as well as whole plant drought responses. Overall, these data present a complex regulatory picture, where G-protein subunits have distinct, tissue-specific roles in regulation of plant drought stress response (Alvarez et al., 2015; Chakraborty et al., 2015; Fan et al., 2008; Nilson and Assmann, 2010).

The stomatal responses of G-protein mutants are studied in detail. The *gpa1* mutant shows wild type (WT) like response to ABA-dependent promotion of stomatal closure, but hyposensitivity to ABA-dependent inhibition of stomatal opening (Wang et al., 2001). The *agb1* and *agg3* mutants also show impaired inhibition of inward K^+ channels in guard cells (Fan et al., 2008), whereas the *agg1* and *agg2* mutants have wild-type like stomatal ABA responses (Chakravorty et al., 2011; Trusov et al., 2008). These data predict that overexpression of specific G-protein genes may lead to better stress tolerance, by making guard cells more responsive to ABA/drought stress. However, over-expression of *AGB1* in wild-type or in the *agb1* backgrounds did not alter ABA-mediated inhibition of the inward K^+ current and the stomata showed ABA sensitivity similar to the WT plants (Fan et al., 2008).

Drought stress (and other abiotic stresses) also causes major spatiotemporal changes in intracellular Ca^{2+} concentration, which acts as an important secondary messenger in ABA-dependant ion channels activation (Huang et al., 2019; Konrad et al., 2018). A theoretical Boolean model of gene expression changes related to the stomatal opening/closing following the removal of ABA or external Ca^{2+}predicts that cytosolic Ca^{2+} oscillation is a deterministic factor for maintenance of stomatal physiology (Albert et al., 2017; Li et al., 2006; Maheshwari et al., 2020). Genetic studies have revealed that AGB1 is required for sensing guard cell Ca^{2+} and

calcium induced release of Ca^{2+} to amplify the signal for stomata closure in the presence of ABA (Jeon et al., 2019). An involvement of extracellular calmodulin (extCaM) and calcium-binding caleosin proteins (RD20/CLO) has also been identified (Brunetti et al., 2021).

In addition to the regulation of specific K^+ and Ca^{2+} channels, a recent study has also shown the involvement of RAPID ALKALIZATION FACTOR1-FERONIA (RALF-FER) signaling module in stomatal movement regulation, where binding of RALF1 peptide ligand to its receptor kinase FER promotes stomatal closure and inhibits stomatal opening. Biochemical studies have shown that AGB1 interacts with FER and the RALF-FER mediated stomatal regulation requires AGB1, as the *agb1* was impaired in such response. Importantly, AGGs and XLGs are also implicated in RALF1-FER dependant stomatal response (Yu et al., 2018).

Another signaling module involved in regulating ABA and stomatal physiology is the interaction of lipid mediated signaling with G-proteins. In Arabidopsis, Phospholipase Dα1 (PLDα1) catalyses hydrolysis of membrane lipids into phosphatidic acid (PA), which is as an important secondary messenger in ABA signaling pathway (Mishra et al., 2006; Roy Choudhury and Pandey, 2016b; Zhao, 2015). In the presence of exogenous ABA, PA produced by PLDα1 activity binds with the PP2C phosphatases, which are the key negative regulator of ABA perception module (Mishra et al., 2006). Incidentally, both PA and PLDα1 interact with GPA1 to mediate ABA inhibition of stomatal opening. Furthermore, recent studies have shown that PLDα1 also acts as a GAP for GPA1 in Arabidopsis and acts in a negative feedback loop where RGS1 inhibits PLDα1 activity, whereas PA produced by PLDα1 binds with RGS1to inhibit its activity, eventually resulting in a dynamic, active GPA1 pool (Roy Choudhury and Pandey, 2017).

G-protein dependent effects of ABA on stomatal guard cells have two more aspects, which play important role in regulating drought responses of the plant. One, AGB1 has also been proposed to promote ABA biosynthesis (Liu et al., 2017). Since the loss of AGB1 function is effectively similar to the loss of entire G-protein function in plants like Arabidopsis (i.e. single Gβ protein that interacts with multiple Gα or Gγ) (Pandey, 2019; Roy Choudhury et al., 2020; Smythers et al., 2022; Urano et al., 2016), one would predict the role of G-proteins in regulating not only ABA signaling but also ABA biosynthesis, during drought stress. Second, G-proteins also regulate key developmental phenotypes such as primary root length and lateral root density as

well as stomatal density. The Arabidopsis *gpa1* mutants have significantly lower and the *agb1* mutant have significantly higher root mass and stomatal density than the WT plants (Chen et al., 2006; Ullah et al., 2003; Zhang et al., 2008). Both these traits will affect the whole plant drought response. Furthermore, the role of specific G-protein subunits and of their core interaction partners such as XLGs, RGS1 and PLDα1 in regulation of stomatal development is exceedingly complex, and mostly unexplored (Pandey, 2019; Roy Choudhury et al., 2020). These intersecting sets of regulations make the prediction of the exact role of G-proteins at the whole plant level difficult. To address this, several studies have evaluated the changes in G-protein-dependent transcriptome (Li et al., 2012; Pandey et al., 2010; Wang et al., 2011), proteome (Alvarez et al., 2015; Gookin et al., 2008; Song et al., 2018; Zhao et al., 2010), metabolome (Jin et al., 2013) and redox-proteome (Smythers et al., 2022) in Arabidopsis. These results suggest that during G-protein dependent, ABA/drought signaling several regulatory pathways intersect, causing key changes in primary and secondary metabolism, photosynthetic efficiency, redox homeostasis and ion balance, eventually leading to the optimal plant response. This complexity of response regulation is also reflected when analysing the whole plant drought response of the G-protein mutants. For example, based on the ion channel activities alone, one would predict that the *gpa1* mutants would have open stomata even under water stress and therefore lower transpiration efficiency (ratio of carbon assimilation and transpiration). However, the plants show higher transpiration efficiency under drought stress and upon exogenous application of ABA (Nilson and Assmann, 2010), likely due to fewer stomata per leaf. Such observations necessitate detailed evaluation of multiple interconnected networks, before specific traits or genes are modified in crop plants.

To evaluate the effects of specific genes in conferring stress tolerance, the *AGG3* gene of Arabidopsis was overexpressed in *Camelina sativa,* an emerging oil seed crop. Constitutive and seed-specific overexpression of *AGG3* resulted in drought tolerance due to higher photosynthetic rate with greater stomatal conductance leading to elevated transpiration rate (Roy Choudhury et al., 2014). The seed specific proteome from these transgenics identified proteins involved in drought tolerance corroborating its role as a positive regulator of plant stress responses (Alvarez et al., 2015). The effects of efficiently managed stress response also translated to better productivity, as seen by an overall increase in biomass, seed size and seed yield, in these

transgenic Camelina plants under greenhouse conditions (Roy Choudhury et al., 2014) and in field trials (unpublished data).

Several studies have demonstrated the roles of different G-protein subunits in regulating drought responses in *Oryza sativa* (rice). The G-protein complex in rice consists of one Gα (RGA1), one Gβ (RGB1) and five Gγ subunits, RGG1, RGG2, DEP1 (RICE DENSE AND ERECT PANICLE 1), GS3 (GRAIN SIZE3) and GGC2 (Perfus-Barbeoch et al., 2004; Sun et al., 2018). Of these, *RGA1*, *RGB1*, *RGG1* and *RGG2* transcripts are upregulated under drought stress, implying their plausible involvement in mitigating this response (Cui et al., 2020). The *d1* mutant of rice, which possesses a non-functional RGA1 protein, exhibits higher stomatal conductance under drought stress with increased photosynthetic rate and a higher root to shoot ratio, suggesting that RGA1 is a negative regulator of drought stress response (Ferrero-Serrano and Assmann, 2016). Further characterization of *d1* mutants in rice cultivars Nipponbare and Taichung revealed improved photosynthesis and CO_2 conductance, corroborating this hypothesis (Zait et al., 2021). Transcriptomic analysis of the *d1* mutant shows several differentially expressed genes related to drought stress response (Jangam et al., 2016), supporting the role of G-proteins in regulating such response at the gene expression level, in addition to the improved stomatal conductance. Use of CRISPR/Cas9 mediated mutagenesis to generate precisely edited G-protein genes in rice and consequent mutant analysis confirmed the results obtained with the *RGA1* gene (Cui et al., 2020). The *rga1* mutants showed better survival rate under induced drought stress. Of the four putative XLGs in rice, *pxlg4* knockout mutant also showed better survival following drought treatment compared to the wild-type (Cui et al., 2020), suggesting the Gα proteins, in general, may act as negative regulators of drought responses in rice. The molecular mechanisms underlying these responses are yet to be determined.

Contrary to the regulation by the Gα proteins, the RGB1 subunit is a positive regulator of drought tolerance in rice. RGB1 has been shown to promote ABA biosynthesis, similar to the role of Arabidopsis AGB1 (Zhang et al., 2015). Genetic analysis shows that the knockdown of *RGB1* causes hypersensitivity to drought stress due to elevated water loss (Zhang et al., 2015). Similar response was seen in different Gγ gene-edited mutants i.e., *rgg1*, *rgg2*, *gs3*, and *ggc2* which exhibited hypersensitivity to drought stress (Cui et al., 2020). Genetic ablation of the Gγ gene (*qPE9-1* allele) in rice is also reported to have conferred drought tolerance due to reduced

water loss and higher stomatal conductance. Moreover, it was shown that *qPE9* represses ABA responsive transcription factors involved in stress tolerance and therefore acts as negative regulator of drought stress in rice (Zhang et al., 2015). It should be noted that the phenotypes of the G-protein mutants in dicot versus monocot plants are distinct- the monocot Gα mutants are of smaller stature, with bushy leaves, a phenotype not seen in the dicot Gα mutant plants (Bhatnagar and Pandey, 2020; Bommert et al., 2013; Cui et al., 2020; He et al., 2013). Complete knockout of monocot *Gβ* gene results in seedling lethality (Utsunomiya et al., 2012; Wu et al., 2020) and consequently, the available data are from the plants expressing lower levels of *Gβ* gene (Gao et al., 2019; Utsunomiya et al., 2012; Wu et al., 2020). In dicots, the complete loss of *Gβ* gene results in multiple phenotypic changes, inherently and in response to a signal, but plants are viable and able to complete their life cycle (Roy Choudhury et al., 2020; Ullah et al., 2003). Monocot plants expressing lower levels of *Gβ* gene also exhibit several morphological differences from the WT plants, such as short stature, bushy and narrow leaves (Utsunomiya et al., 2012; Wu et al., 2020). The extent to which these developmental phenotypes contribute to the overall plant drought responses is yet to be explored.

The role of G-proteins in regulation of drought stress is reported from a few more crops such as pea, tobacco, sugarcane, cucumber and mulberry (Bhardwaj et al., 2020; Liu et al., 2021b; Ramasamy et al., 2021). In most of these cases the results are reported based on the transcript level change in G-protein genes in response to drought stress, overexpression in the heterologous system (e.g., mulberry gene overexpressed in tobacco (Liu et al., 2018)), or based on protein-protein interactions, and the mechanistic knowledge about the signaling pathways involved remains limited (Table 1). Additional studies, exploring the roles of G-proteins in conferring drought tolerance in greenhouse and in field settings are required to fully utilize their potential in generating drought tolerant crops.

4.2 Salinity and osmotic stress

When exposed to high salt, plants experience a combination of osmotic shock due to reduced water availability in the soil and ionic stress imparted by excess Na^+ uptake which eventually interferes with uptake of other ions like K^+ leading to ionic imbalance (van Zelm et al., 2020). The salinity driven osmotic stress attenuates plant growth due to the arrest of cell proliferation in the meristems and results in cell death by ion toxicity (Liu et al., 2015). Notably, the salinity

stress in plants results in accumulation of reactive oxygen species (ROS) which is detrimental to proper biomolecule functioning and restrains plant growth and productivity (Miller et al., 2010). Thus, maintenance of ion homeostasis and detoxification of ROS are the major adaptive responses of plants during salinity stress.

G proteins are implicated in modulating plant's growth during salt stress. Several studies have highlighted the possible involvement of G-proteins in maintaining ion fluxes and scavenging ROS during salinity stress (Liu et al., 2018; Peng et al., 2019). Transcriptomic analysis also revealed that the core G protein subunits Gα, Gβ and Gγ are significantly up-regulated under salt stress in Arabidopsis and other crop plants like rice, rape seeds and peas (Gao et al., 2010a; Jangam et al., 2016; Yadav et al., 2012).

The Arabidopsis *gpa1* mutants exhibit higher tolerance to salt stress compared to the WT plants, in term of seed germination, root-shoot ratio, relative water content and ROS detoxification (Chakraborty et al., 2015), implying that the Gα subunit is a negative regulator of salt stress. The *agb1* mutants, on the other hand, exhibit higher sensitivity to salinity stress, as NaCl treatment results in smaller and chlorotic leaves with an increased Na^+ and reduced K^+ in the roots leading to ion imbalance (Colaneri et al., 2014). Molecular characterization of *agb1* showed that the mutant was compromised in translocation of Na^+ from root to shoot resulting in altered Na^+ fluxes. Genes involved in Na^+ homeostasis are significantly downregulated in *agb1* (Ma et al., 2015b). The hypersensitivity of *agb1* mutant to salinity stress was also correlated with the reduced peroxidase activity required for ROS detoxification (Ma et al., 2015b). Similar to *agb1*, *xlg123* triple and *agg123* triple mutants also show hypersensitivity to salinity stress and exhibit smaller and chlorotic leaves when grown in the presence of high NaCl (Colaneri et al., 2014; Roy Choudhury et al., 2020). While AGGs are implicated in salt stress tolerance by activating ROS detoxification, XLGs do so by interacting with salt inducible zinc finger transcription factors SZF1 and SZF2. It has been proposed that AGB1 interacts with XLGs to promote plant growth during salt stress through expression of SZF1 and SZF2 (Liang et al., 2017). These findings highlight the role of AGB1(or XLG.AGB1.AGG trimer) as a positive regulator of salinity stress by ROS detoxification and maintaining ionic balance, although the detailed underlying molecular mechanisms are only beginning to be uncovered.

Maintenance of cell wall integrity during salinity/osmotic stress is crucial for plants to withstand the turgor pressure (Rui and Dinneny, 2020; Vaahtera et al., 2019). A key component of the maintenance of the cell wall integrity is the recently identified LRX-RALF-FER signaling module in Arabidopsis (Feng et al., 2018). The leucine rich repeat extensin (LRX) crosslinks with cell wall pectin. FER also binds pectin through its extracellular malectin-binding domain. Cell type specific increases in Ca^{2+} fluxes are also required to maintain cell wall integrity through crosslinking of pectins in FER-dependant fashion. These interactions are disrupted under salt stress and the cell wall integrity is lost in *fer* loss-of-function mutant. Because AGB1 has been identified as a key interactor of FER (Yu et al., 2018), an AGB1/LRX-RALF-FER signaling module may also be involved in pectin crosslinking associated cell wall damage during salinity stress (Feng et al., 2018; Zhao et al., 2018).

A forward genetic screen in rice identified a novel mutant allele of *RGA1, sd58,* which showed better salt tolerance due to reduced accumulation of ROS, consistent with higher enzymatic activity of ROS detoxification enzymes (Peng et al., 2019). Quantitative proteomics identified differentially expressed proteins involved in regulation of photosynthesis, metabolic processes and ROS homeostasis in *sd58*. Similarly, CRISPR/Cas9 edited Gα mutants in rice, *rga1-1* and *rga1-2* also exhibit better survival following salinity stress (Cui et al., 2020). These mutants showed delayed leaf senescence, lower chlorophyll degradation and reduced electrolyte leakage from the cytoplasm during salt stress (Cui et al., 2020). The *Gα* subunit, CT2 (COMPACT PLANT2) in maize is an important regulator of agronomic traits including upright leaf, higher spikelet density and kernel row number (Bommert et al., 2013). The *ct2* null mutant exhibited better salt tolerance with reduced electrolyte leakage from the cytoplasm, delayed leaf senescence and chlorophyll degradation, similar to *rga1* in rice (Urano et al., 2014). All these studies support the role of Gα proteins as a negative regulator of salt stress response in plants. In contrast, the results obtained from the overexpression of the *Gα* genes in pea led to improved salt tolerance (Misra et al., 2007), whereas knockdown of the *Gα* gene expression in cucumber led to hypersensitivity to salt stress with increased leaf wilting and reduced water content (Yan et al., 2020), suggesting their role as positive regulators. The extent to which these results are due to different stress conditions, age of the plants or additional experimental factors, is not known.

Similar to what was observed with the drought stress response, the Gβ and Gγ proteins seem to be positive regulators of salinity stress in rice. Overexpression of *RGB1* resulted in better salt tolerance with reduced electrolyte leakage and higher chlorophyll content (Biswas et al., 2019). At the molecular level, the better salt tolerance was correlated with increased expression of ROS detoxification enzymes like superoxide dismutase. Concurrent over-expression of *RGB1* and *RGG1* in rice improved salt tolerance by enhanced expression of stress responsive genes and better management of ROS (Swain et al., 2019). However, *rgb1* mutant generated by CRISPR/Cas9 mutagenesis showed better survival following salinity treatment (Cui et al., 2020), confounding the previous observations. The non-canonical Gγ subunits in rice, DEP1 and GS3 may also act as negative regulator of salt stress as *gs3* and *dep1* mutants showed better survival rate following salinity treatment and both the mutants had higher yield (Cui et al., 2020). In a few other crop species where the roles of G-proteins in salt stress has been analysed, a similar confounding picture emerges- the overexpression of a *Gβ* gene in pea led to no effect (Misra et al., 2007), whereas the overexpression of mulberry *Gβ*, *Gγ1* or *Gγ2* genes led to increased salt tolerance (Liu et al., 2018; Liu et al., 2017). As mentioned earlier, the contrasting results could be due to the experimental conditions, or due to the inherent nature of the G-protein complex regulation (discussed in the next sections), and need further confirmation.

4.3 Temperature stress

Extreme hot and cold temperatures affect both the vegetative and reproductive phases of plant life cycle resulting in a significant decrease in crop productivity (Zhang et al., 2022). At the molecular level, heat stress adversely affects various biomolecules resulting in altered membrane fluidity leading to the loss of cell membrane integrity, reduced protein synthesis, improper protein functionality due to their aggregation and altered enzymes kinetics (Niu and Xiang, 2018). The morphological changes include delayed seedling establishment with an overall reduction in plant growth rate, smaller leaves, early senescence and abscission, elongated hypocotyl, petiole and damaged fruit (Bita and Gerats, 2013). Heat stress also affects reproductive development in plants such as reduced pollen and ovule viability resulting in poor fertilization, slower pollen tube elongation, improper floral organ development and closed floral buds with reduced seed vigor (Endo et al., 2009; Kumar et al., 2013; Snider et al., 2011). Among physiological changes, reduction in the rate of photosynthesis, respiration and transpiration is

more evident during heat stress, accompanied with an overall increase in the reactive oxygen species (ROS) and phytohormone production (Kumar et al., 2012; Yin et al., 2008).

In response to cold stress, plants need to synthesize cryoprotectants such as soluble sugars, proline and cold-resistance proteins to protect themselves from the freezing temperatures by regulating osmotic potential,avoiding ice crystal formation and providing stability of the cell membrane (Kaplan and Guy, 2004). Calcium channels have been involved in low-temperature sensing in plants (Knight and Knight, 2012). Ca^{2+}, along with other secondary messenger molecules such as ROS and NO are involved in regulating plant response to cold stress (Knight and Knight, 2012; Zhao et al., 2009), although the downstream events involved in the cold signalling pathway are poorly understood.

As with other types of abiotic stresses, the involvement of G-proteins has been shown in response to temperature stress tolerance. In Arabidopsis, these responses are mostly evaluated at the transcriptomic level, where a number of transcripts related to temperature stress were differentially expressed in *gpa1* mutants (Chakraborty et al., 2015). The *gpa1* mutants exhibit significantly increased tolerance to cold stress and a subtle increased tolerance to heat stress, corroborating the transcriptomics data (Chakraborty et al., 2015). However, in contrast to other stresses, where their response mechanisms have been characterized, to an extent, no mechanistic data exist for temperature stress response of the G-protein mutants in Arabidopsis.

Transcriptional regulation of different G-protein subunits themselves has been reported in rapeseed (*Brassica napus*). The *Gα* (*BnGA1*), *Gβ* (*BnGB1*) and *Gγ* (*BnGG2*) transcripts show downregulation in response to heat and cold stresses (Gao et al., 2010a; Gao et al., 2010b), suggesting that the G-protein subunits may act as negative regulators of temperature stress responses in plants. This was supported by the heterologous overexpression of a wheat Gβ protein, TaGPBL in Arabidopsis, which causes reduced plant growth at 16 °C. These plants also show reduced expression of cold-inducible genes and lower activity of ROS scavengers, compared to WT plant, corroborating the role of Gβ proteins as a negative regulator of temperature stress signaling (Dong et al., 2019).

In other species, such as Chinese pear (*Pyrus pyrifolia*) six out of eight *Gα* genes were upregulated in response to high temperature in leaves (Chen et al., 2022). Similarly, the transcript levels of pea *Gα* and *Gβ* genes showed higher expression after heat treatment (Misra et al., 2007). In addition, transgenic tobacco plants constitutively expressing *PsGα* or *PsGβ* showed

tolerance to heat stress when tested by leaf-disk senescence assay and germination/growth of T1 seeds/seedlings (Misra et al., 2007). Further characterization of tobacco plants overexpressing *PsGβ* suggest that the heat stress response is mediated by nitric oxide (NO)-induced stomatal closure during heat stress (Bhardwaj et al., 2020), and may also include mitogen activated protein kinase (PsMPK3).These results suggest a positive role of G-proteins during temperature stress response, in contrast to what has been suggested for Arabidopsis. Studies in tomato plants expressing altered level of *Gα* gene by *RNAi* and overexpression approaches also support a positive role of these proteins in cold stress tolerance (Guo et al., 2020). *LeGPA1-RNAi* and *LeGPA1-OX* plants exhibit reduced and improved tolerance to cold stress, respectively, compared to the WT tomato plants. The *LeGPA1-OX* plants showed higher activities of the antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) leading to lower accumulation of H_2O_2 and O^{2-}. Moreover, there was an upregulation of the transcripts involved in cold signaling, which resulted in an increased levels of proline and soluble sugar that protect against cellular damage (Guo et al., 2020).

The involvement of G-proteins in controlling temperature stress in rice has been studied at multiple levels. Transcriptomic analysis of the *d1* mutant identified hundreds of differentially expressed transcripts related to temperature stress tolerance. Specific G-protein subunits themselves are also regulated at the transcript level and show altered expression in response to cold and heat stress. As with other stresses, the regulation seems to be complex. For example, the transcript level of *RGA1* was reduced in response to elevated temperature. In contrast, the *RGB1, RGG1* and *RGG2* transcripts were upregulated in response to both heat and cold stress (Yadav et al., 2013; Yadav et al., 2014). The G-protein dependent cold stress response in rice has been also linked to a quantitative trait locus COLD 1 (Chilling-tolerance divergence 1), a homolog of Arabidopsis GTG proteins, which interact with GPA1 (Pandey et al., 2009). COLD1 interacts with rice RGA1, and affect its GTPase activity and calcium channel activation. Overexpression of COLD1 significantly improved chilling tolerance, whereas its downregulation was reported in cold-sensitive rice lines (Ma et al., 2015a). Overexpression of the *RGB1* gene in rice also led to improved heat stress tolerance, potentially via effective mitigation of ROS and activation of heat shock proteins (Biswas et al., 2019).

One of the rice type III Gγ proteins, GS3, is recently identified as the causal gene underlying the quantitative trait locus for heat stress tolerance, thermotolerance 2, TT2 (Kan et al., 2022). Heat-

treated plants with the natural allele of disrupted TT2 function exhibited a reduction in wax content, therefore an enhanced thermotolerance in comparison with plants carrying the functional TT2 allele. The transcription factor SCT1 (Sensing Ca^{2+} Transcription factor 1) is a calmodulin 2 (CaM)-interacting Ca^{2+} decoder, that negatively regulates the OsWR2 gene (Wax Synthesis Regulatory 2). The CaM–SCT1 interaction was affected in plants with disrupted TT2, revealing that the G-protein TT2 regulates thermotolerance by mediating heat-triggered Ca^{2+} signaling and Ca^{2+}/CaM-dependent suppression of SCT1 transcriptional activity to control wax biosynthesis in rice (Kan et al., 2022).

The roles of G-proteins in mediating temperature stress has been evaluated in a few other species. In wheat, the Gα gene, *GA1-D*, and two of the Gγ genes, *Gγ2-B* and *Gγ2-D*, were significantly upregulated by cold and heat stresses (Gawande et al., 2022). In cucumber, a type III Gγ protein, CsGG3.2, has been shown to be involved in the regulation of cold stress tolerance by modulating the CBF (Cold Binding Factor) signaling module and resulting in increased activities of antioxidative enzymes and consequently decreased production of ROS, reduced membrane lipid peroxidation after cold stress (Bai et al., 2018). A recent study in sugarcane (Saccharum spp.), implies a role of G-protein signaling in stress responses. The sugarcane GPCR-like protein (ShGPCR1), a homolog of the Arabidopsis GCR1, was upregulated by cold, drought and salinity stresses (Ramasamy et al., 2021). GCR1 is a known interactor of GPA1 and regulates stress response in Arabidopsis, potentially via G-protein signaling (Pandey and Assmann, 2004). Constitutive overexpression of *ShGPCR1* in sugarcane conferred tolerance to multiple abiotic stresses and showed activation of multiple cold-stress marker genes such as NAC23 (NAM/ATAF1/2/CUC), CBF2 (Cold Binding Factor 2), ScADH3 (Alcohol Dehydrogenase 3), as well as several drought and salinity marker genes (Ramasamy et al., 2021).

4.4 Heavy metal stress

Heavy metals such as cadmium or arsenic impose significant stress on plants under specific growth environments. Especially, Cd due to its chemical similarity to metal co-factors such as Zn, Fe, and Ca, can inactivate and denature proteins by binding to free sulfhydryl groups (DalCorso et al., 2008). Plants typically cope up with Cd toxicity by its sequestration into the vacuoles, a process that largely involves cysteine (Cys)-rich proteins that can chelate the heavy metals (Freisinger, 2008). Several studies have demonstrated the role of the unique type III Gγ proteins in Cd

tolerance. Overexpression of rice DEP1 (a type III Gγ protein) in heterologous systems, such as in yeast and in Arabidopsis resulted in tolerance to high levels of Cd (Kunihiro et al., 2013). Similarly, the overexpression of Arabidopsis *AGG3* gene in Camelina improved Cd tolerance, which was also supported by the quantitative proteomics analysis, where several proteins related to heavy metal toxicity were differentially abundant (Alvarez et al., 2015). The response to proposed to be mediated via the Cys-rich C-terminal region of these proteins.

4.5 Other atmospheric stresses

In addition to their relatively well documented roles in droughts, temperature and salinity stress, the involvement of G-proteins has been demonstrated in other adverse atmospheric conditions such as UV-B radiation and ozone (O_3) (He et al., 2013; Joo et al., 2005).

In plants, the high energy enriched UV-B radiation causes thickening of leaves and cuticular wax layers and reduction of photosynthetic efficiency, plant growth, and pollen fertility (Caldwell et al., 2007). Stomatal regulation is central to the plants' response to UV-B. Exposure to UV-B results in increased production of reactive oxygen and reactive nitrogen species in the stomatal guard cells, which ensures stomatal closure under high light/UV-B radiation (Jansen and van den Noort, 2000). ABA elicits the production of ROS including H_2O_2 under such conditions (He et al., 2013). In Arabidopsis, plasma membrane bound NADPH oxidases RbohD and RbohF are involved in generation of H_2O_2 in an ABA-dependant manner (Kwak et al., 2003). Being an important mediator of stomatal ABA response, *gpa1* mutant is compromised in stomatal closure under high UV-B treatment due to reduced H_2O_2 and NO production. However, exogenously added H_2O_2 and NO can rescue the stomatal closure defect of *gpa1* suggesting that GPA1-mediated signaling is upstream of UV-B-mediated H_2O_2 and NO production (He et al., 2013). The involvement of other G-protein subunits in UV-B response is largely unknown.

In addition to UV-B, ozone is also harmful for plants as it can enter through stomata (Torsethaugen et al., 1999) and generate oxidative stress intracellularly, resulting in massive cellular damage (Joo et al., 2005). Elevated O_3 affects crop productivity by reducing yield and quality. For example, wheat and rice exposed to high levels of O_3 produced significantly smaller grains with decreased starch content, increased protein and nutrient (P, K, Mg, Ca, Zn, Fe) contents, affecting the grain texture and its baking properties (Broberg et al., 2015; Ueda et al., 2015). Similar to UV-B radiation, O_3 also induces ROS production primarily in the chloroplasts of the stomatal guard cells

(Evans et al., 2005). The signal is transmitted to the adjoining cells where extracellular ROS act as a molecular trigger for generation of intracellular ROS production through membrane localized NADPH oxidases *RbohD* and *RbohF*. The *gpa1* and *agb1* mutants have reduced and increased sensitivities, respectively, to O_3-induced damage. It was proposed that the Gβγ complex mediates the early chloroplastic oxidative burst, while the Gα induces the late ROS production that leads to the activation of the membrane-bound NADPH oxidases, necessary for transmitting the ROS signal and trigger cell death (Joo et al., 2005). Ozone and UV-B radiation stress mediated effects may be correlated, as the depletion of the stratospheric ozone layer exacerbates the harmful effect of UV-B on crop productivity. However, the role of G-protein subunits in ozone and UV-B tolerance in crop plants largely remains unknown.

5. Signaling modules regulated by G-proteins in regulation of stress responses

The overall description of the role of G-proteins in mediating abiotic stress responses in plants presents a complex picture. However, a closer examination of several of these responses supports scenarios where G-proteins potentially affect a few fundamental signaling modules, which by interconnecting with discrete signaling networks may result in signal specific responses (Figure 2). For example, the role of G-proteins in affecting ABA signaling pathways places them in a central position to regulate almost all abiotic stress responses (Fan et al., 2008; Liu et al., 2021a; Yu et al., 2018; Zhang et al., 2015). G-proteins regulate signaling pathways of several other phytohormones, which intersect and feedback into ABA synthesis and signaling networks (Alvarez et al., 2015; Bhardwaj et al., 2020; Smythers et al., 2022; Zhang et al., 2015). ABA responses are also connected to ROS production (Bohmer and Schroeder, 2011; Mittler and Blumwald, 2015). Regulation of ROS levels is central to the normal growth, development and productivity of plants. By their ability to affect the ROS levels, either by their interactions with NADPH oxidases, or via the high Cys containing regions of the type III Gγ proteins, or other unknown mechanisms, G-proteins have the ability to affect several of these stress responses (Bai et al., 2018; Guo et al., 2020; Liu et al., 2017). Another module potentially involves the change in membrane potential and dynamics as well as in plasma membrane composition, which can eventually affect fundamental cellular properties and response, as well as ion channel activities (Assmann and Jegla, 2016; Huang et al., 2019; Kim et al., 2010; Munemasa et al., 2015). G-proteins are known to interact with and regulate several phospholipases, sphingosine

phosphatases and kinases, potentially affecting multiple aspects of lipid biosynthesis and signaling (Coursol et al., 2003; Roy Choudhury and Pandey, 2016b; Zhao and Wang, 2013). Additionally, developmental regulations, such as the stomatal density, which are key to plants interaction with its environment, interactions with intracellular membrane systems, such as ER biogenesis and regulation and potentially cell wall composition also contribute to the G-protein-dependent responses (Feng et al., 2018; Roy Choudhury et al., 2019; Roy Choudhury and Pandey, 2016b; Rui and Dinneny, 2020). Even though the cause and effect relationships of these signaling/developmental modules is not clear, future research geared towards identifying these will certainly result in critical knowledge needed to harness the power of these proteins in generating stress tolerant plants.

6. The challenges and future perspectives

One of the biggest challenges in synthesizing the available data on the roles of G-proteins in plant abiotic stress responses is the seemingly random, often contrasting phenotypes observed in various studies. This is confounded primarily by the inherent composition and signaling mechanism of G-proteins. An initial level of complexity is introduced at the level of trimer composition itself. In plants such as Arabidopsis and rice, that have simpler repertoire of G-proteins, a single Gβ protein can interact with one of multiple Gα or Gγ proteins. When studying the effect of the loss of an individual Gα or an individual Gγ gene (e.g., *gpa1, rga1, dep1* mutants), it is not clear if the effects observed are due to the loss of this individual protein or an effect of a varied stoichiometry between different subunits (Pandey, 2019; Roy Choudhury et al., 2020; Urano et al., 2016). For example, the phenotype of a *gpa1* mutant could be due to the loss of GPA1 function or due to an altered or availability of AGB1 for XLG proteins or due to constitutive signaling by freed Gβ (Roy Choudhury and Pandey, 2022). In such situations, the effects of the loss of a gene function may not be exact opposite of the protein overexpression. The situation becomes significantly more complex when studying plants with higher numbers of each of the G-protein subunits such as soybean or wheat. Moreover, the protein complex is trimeric, but it is active when the trimer is dissociated. Both Gα and Gβγ can be functional signal transducers, but can also affect each other's availability/localization (Chakravorty and Botella, 2007; Trusov et al., 2007; Wang and Botella, 2022). Therefore, results with gain- or loss-of-function of an individual protein should be interpreted cautiously. The modular structures of

specific proteins also adds to this complexity. For example, the C-terminal of the type III Gγ protein has been proposed to be an inhibitor of its N-terminal Gγ-like domain (Botella, 2012; Tiwari and Bisht, 2022). In this case, mutations resulting in removal of only the C-terminal may actually result in a highly active Gγ protein, similar to its overexpression. Examples of such effects have been seen during grain size regulation in rice by the *GS3* gene, where site-specific mutations in the same gene may result in smaller or longer grains (Botella, 2012; Cui et al., 2020; Fan et al., 2009; Mao et al., 2010).

Another level of complexity is added by the potential tissue or organ-specific roles of these proteins. Although such studies are limited mostly to Arabidopsis, it appears that significant differences exist. For example, the Arabidopsis *gpa1* and *agb1* mutants show hyposensitivity to ABA during stomatal opening responses, these same mutants are hypersensitive to ABA during seed germination (Fan et al., 2008; Smythers et al., 2022; Yu et al., 2018). It is therefore important to consider the plant's response to specific signals in totality, not only in a specific tissue type.

A further key point to consider is the experimental designs themselves. The plant growth condition, plant age, stress treatment conditions, severity of stresses, time etc. all vary considerably between experiments. Many of these are performed in heterologous systems, most under laboratory conditions, which has little relevance to actual plant growth in fields. The timings and methodologies of how plants are subjected to stress conditions is also important, but rarely considered. For example, stress experienced by the plants at their vegetative growth stage maybe tolerated better than the stress experienced at the time of flowering, or seed filling. Similarly, the way plants perceive these stresses needs to be optimized for each species. A rice seedling, submerged in water during early growth may not experience same severity of heat stress compared to a wheat seedling growing at a similar higher temperature. Finally, plants growing in fields experience several stresses simultaneously, and thus will respond differently than what is assessed with plants grown in control laboratory conditions, subjected to one specific stress at a time.

In summary, it is obvious that the global climate change has already exacerbated the harmful effects of various abiotic stresses in crop plants, drastically reducing their overall productivity worldwide. It is of utmost importance to design/breed stress resilient crops to meet the needs of

the future generation. The information obtained so far places the G-proteins in a central position to serve this role. However, an integrated approach combining the current 'cause/effect' information with precise genome-editing technologies, multi-omics analysis and modelling, extensive crop physiology, agriculture economics and management is required to apply it directly to the crops of interest, in field-settings, to enable food security.

Conflict of interest

The authors have no conflict of interest related to the work described in this manuscript.

Author Contributions

PM, DRTR and SP wrote the review.

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Figure legends

Figure 1. Heterotrimeric G-protein signaling components and mechanism. **(A)** Domain architecture of G-protein signaling components in plants. Gα proteins have an N-terminal myristoylation site followed by helical domain and C-terminal RAS like domain. The plant-specific XLG proteins possess an extra-large domain of unknown function fused with the canonical G-like domain. The N terminal domain has a nuclear localization signal (NLS), and a cysteine (Cys)-rich region. Gβ proteins have an N-terminal coiled-coil domain and seven WD repeats containing domain. The Gγ subunits are classified into type-I, type-II, and type-III subtypes. Type-I Gγ (canonical) proteins have the Gγ domains that form interaction with the coiled coil domain of Gβ subunit and a C terminal prenylation motif for its membrane anchorage. Type-II Gγ proteins have Gγ domains but lack the prenylation motif. Type-III proteins have the Gγ domains followed by a transmembrane domain and Cys-rich region of variable length. A prototypical GPCR protein with 7 transmembrane regions is also identified in plants (e.g. GCR1 from Arabidopsis). The plant RGS proteins have an N- terminal seven transmembrane domain similar to GPCRs fused with the C-terminal RGS domain. **(B)** Basic G-protein signaling mechanism in plants. The core heterotrimeric G-protein complex comprises of Gα, Gβ and Gγ subunits. The signaling complex shuttles between inactive Gα-GDP and active Gα-GTP forms. Conventionally, the GDP to GTP exchange reaction is catalyzed by GPCR, which acts as guanidine nucleotide exchange factor (GEF). In plants, no such GEF is identified, to date. A phosphorylation-dependent mechanism may be operative during plant G-protein signaling. The Gα subunit has an inherent GTP hydrolysis

activity, which is stimulated by the GTPase activity accelerating protein (GAP), RGS1. RGS1 inhibits PLDα1, whose product phosphatic acid (PA) in-turn inhibits RGS1 and regulates the GAP activity of Gα subunit. Once activated, the Gβγ obligate dimer dissociates from Gα-GTP subunit and both can activate downstream signaling through various effectors.

Figure 2. Signaling pathways involved in G-protein mediated abiotic stress responses in plants. Involvement of abscisic acid (ABA), Ca^{2+}, reactive oxygen species (ROS), rapid alkalization factor-Feronia (RALF-FER), lipid mediated signaling and mitogen activated protein kinase (MAPK) modules has been shown during G-protein modulated abiotic stress signaling pathways. The cause/effect relationships between these modules is not fully elucidated (depicted as double arrowheads).