Physiology and Molecular Biology of Plants
https://doi.org/10.1007/s12298-024-01428-7

REVIEW ARTICLE

Agronomic potential of plant-specific Gγ proteins

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Received: 9 August 2023 / Revised: 17 January 2024 / Accepted: 28 February 2024

Abstract

The vascular plant-specific type III Gγ proteins have emerged as important targets for biotechnological applications. These proteins are exemplified by Arabidopsis AGG3, rice Grain Size 3 (GS3), Dense and Erect Panicle 1 (DEP1), and GGC2 and regulate plant stature, seed size, weight and quality, nitrogen use efficiency, and multiple stress responses. These Gγ proteins are an integral component of the plant heterotrimeric G-protein complex and differ from the canonical Gγ proteins due to the presence of a long, cysteine-rich C-terminal region. Most cereal genomes encode three or more of these proteins, which have similar N-terminal Gγ domains but varying lengths of the C-terminal domain. The C-terminal domain is hypothesized to give specificity to the protein function. Intriguingly, many accessions of cultivated cereals have natural deletion of this region in one or more proteins, but the mechanistic details of protein function remain perplexing. Distinct, sometimes contrasting, effects of deletion of the C-terminal region have been reported in different crops or under varying environmental conditions. This review summarizes the known roles of type III Gγ proteins, the possible action mechanisms, and a perspective on what is needed to comprehend their full agronomic potential.

Keywords AGG3 · DEP1 · GS3 · Heterotrimeric G-proteins · Stress · Type III Gγ proteins

Introduction

We are living through an unprecedented period of population growth, climate change and geo-political crises. The world population is expected to increase to 10 billion people by the year 2050. To put it in the context of population growth rate, it took almost 150 years for the population to increase from 1 billion (in 1800) to 2.5 billion (in 1950), but the last 75 years have already seen the tripling of the population to over 8 billion today (Ritchie et al. 2023). This is accompanied by similar changes in global average temperature, which is now changing at a faster rate than at least over the past 1000 years (IPCC 2023). The changing lifestyle and recent pandemic have also added to the global hunger crisis. The number of people facing, or at risk of, acute food insecurity has increased from 135 million in 53 countries pre-pandemic, to 345 million in 79 countries in 2023 (WFP 2023). Several indices of growth and climate change models project that the current agricultural practices will be insufficient to fulfil the demand for food and feed for the world in the future (Statista 2022; IPCC 2023; WFP 2023; Ritchie et al. 2023). Incidentally, these glooming projections are eerily similar to the predictions made in 1950s and 1960s. At a global food meeting in 1959, Dr. Forrest F. Hill of the Ford Foundation had mentioned "*At best the world outlook for the decades ahead is grave; at worst it is frightening*". In 1968, Paul Ehrlich's best seller book, The Population Bomb, stated that "*the battle to feed all of humanity is over. In the 1970s hundreds of millions of people will starve to death in spite of any crash programs embarked upon now. At this late date nothing can prevent a substantial increase in the world death rate*" (Eherlich 1968). We now know that these predictions did not come to pass because of scientific advances that transformed global agriculture, especially in staple crops like wheat, rice, and maize (Evenson and Gollin 2003; Pingali 2012; Poehlman 2007; Hazell et al. 2010). To continue this trend of improved agricultural practices and crop production between now and 2050, novel biotechnological approaches are critically needed in combination with better management of land, resource, and crops in an environmentally sustainable manner (Annan 2003).

Research in the past decades has identified several gene targets that have the potential to significantly improve crop

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productivity (Bailey-Serres et al. 2019; Nowicka 2019), but their widespread agricultural applications remain limited. One of the reasons is that in general, the desired effects of the target genes are evaluated under specific conditions, or by applying one specific set of variables (*e.g.,* temperature, salinity, or nutrient stress), which is hardly ever the condition during real-life plant growth. Additionally, most agricultural traits are quantitatively controlled, and are a net effect of multiple causal genes or loci. In situations where one specific trait is controlled by a single gene, *e.g., SUB1* gene for submergence tolerance in rice (Fukao et al. 2006; Xu et al. 2006), and where the effects of specific gene manipulations have been obvious in field settings, phenomenal improvements in yield have been recorded (Emerick and Ronald 2019; Alam et al. 2020; Mohapatra et al. 2021; Panda et al. 2021; Kuanar et al. 2023). Examples such as *SUB1* imply that the agronomic potential of our crops is not yet fully realized. At the other end of the spectrum, we have situations where a specific target gene can affect more than one plant trait, such as certain developmental transitions and response to exogenous stresses. Therefore, a deeper understanding of the molecular, cellular and developmental pathways by which plants respond to and interact with biotic and abiotic factors in their environment, while maintaining growth, nutrient and water use efficiency and fitness is fundamental to their utilization for future crop engineering (Bailey-Serres et al. 2019; Nowicka 2019). The few successful examples have also highlight that the integration of these empirical mechanistic data with the analysis of existing genetic variation in natural populations, and modern genome-scale breeding technologies will be essential for effective implementation of such knowledge.

G-proteins as agronomic targets

One group of proteins that have received a lot of attention in recent years for their potential agronomic importance are the type III Gγ proteins. Type III Gγ proteins are constituents of the heterotrimeric G-protein complex in plants. As evident by the name, heterotrimeric G-proteins are composed of three dissimilar subunits, Gα, Gβ and Gγ. G-proteins are major conduits for signal transduction in eukaryotes, ranging from yeast and human to algae and plants. The proteins typically act as a bimodal molecular switch to integrate signals from endogenous or external environments to cellular responses (Pandey 2019). When the Gα protein is in GDP-bound form, the complex exists as a heterotrimer (GDP-Gαβγ), and represents their inactive state (Fig. 1). Upon signal perception by a cognate receptor, the GDP on Gα is replaced by GTP and the trimer dissociates into GTP-Gα and Gβγ. Both these dissociated entities can interact with several downstream effectors to transduce the signal, representing their active signaling state. The Gα protein is also a GTPase, which hydrolyzes the bound GTP to GDP, regenerating GDP-Gα, which reassociates with Gβγ to restore the inactive trimeric complex (Pandey 2019; McCudden et al. 2005; Siderovski and Willard 2005; Oldham and Hamm 2008). The GTPase activity of Gα proteins is aided by the GTPase activating proteins (GAP) such as regulator of G-protein signaling (RGS). The basic biochemistry of G-protein components and their interactions are conserved across phyla. In plants, G-proteins regulate almost all aspects of growth and development and response to environmental stresses, as summarized in several recent reviews (Pandey 2019; Majumdar et al. 2023; Zhao et al. 2022; Tiwari and Bisht 2022; Ofoe 2021; Maruta et al. 2021; Jose and Roy Choudhury 2020; Cui et al. 2020; Zhong et al. 2019; Wang et al. 2019; Hackenberg et al. 2013). In this review I will focus specifically on the roles of type III Gγ proteins, their potential in imparting useful agronomic traits and their complex regulation.

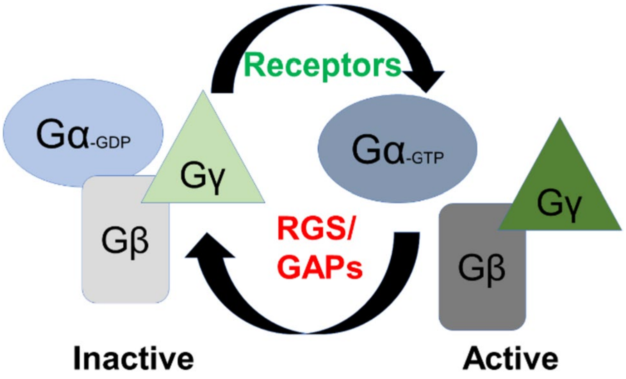

Fig. 1 Switch like signaling mechanism of heterotrimeric G-proteins. RGS refers to the regulator of G-protein signaling, which acts as a GTPase activity accelerating protein (GAP)

In contrast to other organisms, plants have different types of Gγ proteins, classified as Type I, II and III (or group A, B and C) (Chakravorty et al. 2011; Roy Choudhury et al. 2011; Thung et al. 2012). The type I Gγ are canonical proteins similar to the Gγ proteins found in non-plant systems. These are 100–120 aa proteins, represented by Arabidopsis AGG1 and AGG2, rice RGG1 or soybean SGG1-4 (Roy Choudhury et al. 2011). The type II (or group B) Gγ proteins are nearly identical to the type I Gγ proteins except for the lack of a signature C-terminal prenylation motif, required for their plasma membrane localization. Homologs of this protein are missing in Arabidopsis (in all Brassicaceae) but are represented by RGG2 in rice and SGG5-7 in soybean (Roy

Choudhury et al. 2011). Type III (or group C) Gγ proteins, represented by Arabidopsis AGG3, rice GS3, DEP1, GGC2, and soybean SGG8-10, are found only in vascular plants (Chakravorty et al. 2011; Roy Choudhury et al. 2011). A detailed evolutionary analysis of the G-protein components in the plant and algal lineages confirmed the prevalence of these proteins in gymnosperms and angiosperms, and the absence in all other plant groups, although when these diverged from the canonical Gγ proteins remains unresolved (Mohanasundaram et al. 2022).

The type III Gγ proteins have several unique features. These are at least twice as large as the type I or type II proteins and have a modular architecture. The N-terminal region of the proteins is similar in size and sequence to type I and II Gγ proteins and is referred to as Gγ-like domain or the GGL domain (Fig. 2). This region interacts with the Gβ proteins, to form the heterotrimeric G-protein complex. The GGL domain is attached to an extended C-terminal region of varying lengths, with a proposed transmembrane region (Wolfenstetter et al. 2015). The C-terminal region is extremely rich in amino acid Cysteine (Cys), which may constitute 35–40% of this region (Roy Choudhury et al. 2011). This makes the type III Gγ proteins one of the most Cys-rich proteins in nature. Another noticeable feature of these proteins is their copy number in plant genomes. Unlike other G-protein subunits, which are usually present in a single copy in diploid genomes, the type III Gγ proteins are present in multiple copies (Fig. 3). Additionally, when present in multiple copies, the GGL domain of different proteins is highly similar in length and at the sequence level, but the C-terminal region is variable in length and shows little sequence similarity (Fig. 2). For example, the rice proteins GS3, GGC2, and DEP1 have similar GGL domains (114, 112 and 120 amino acids, respectively), but their Cys-rich regions are of variable lengths, corresponding to 117, 223, and 306 amino acids,

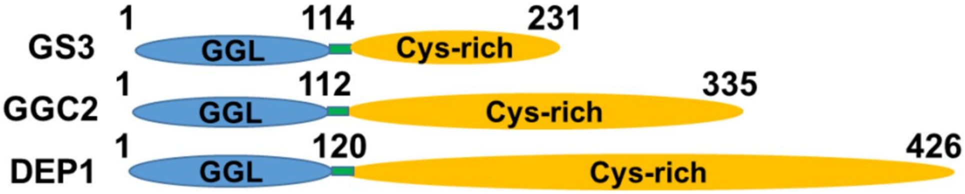

Fig. 2 The type III Gγ proteins of rice. The Gγ-like GGL domain is highly similar between the three proteins but the C-terminal cysteine rich region is of variable lengths

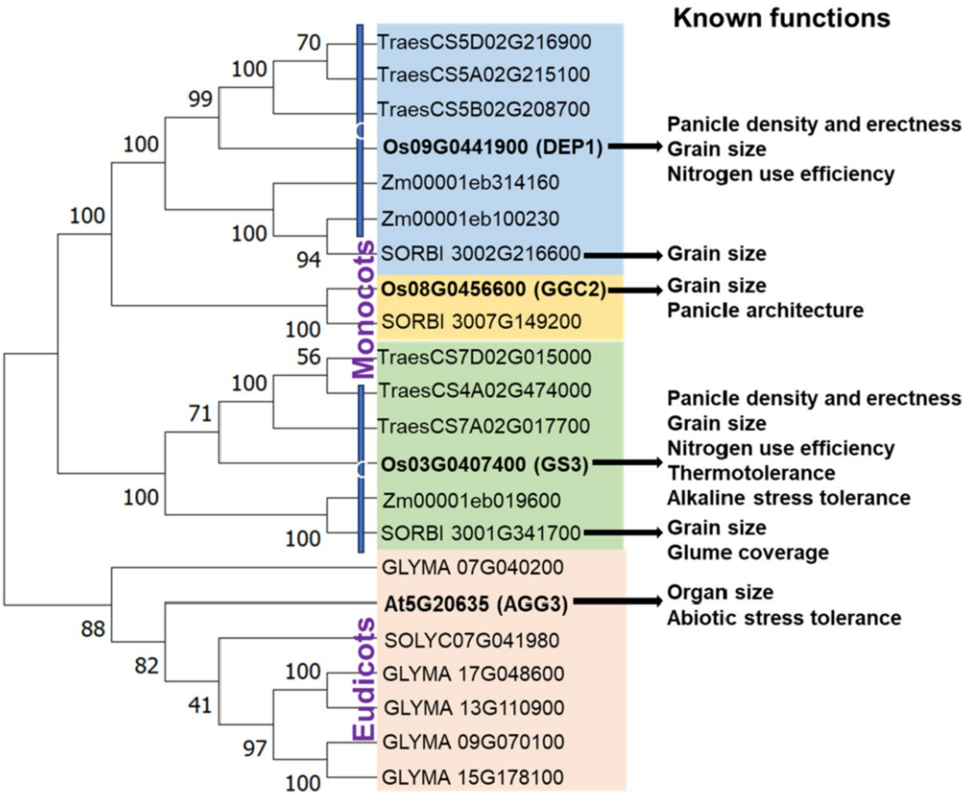

Fig. 3 Evolutionary analysis of type III Gγ proteins from crop plants. The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model. The tree with the highest log likelihood (−6759.81) is shown. Evolutionary analyses were conducted in MEGA11. Eudicot and monocot proteins form distinct groups. The monocot proteins are further subdivided in three groups represented by rice DEP1, GGC2 and GS3, based on their sequence similarities and the length of the C-terminal region. Any known functions of the proteins are also listed

respectively. Similar extension of the C-terminal region is observed in other plant species and the proteins form distinct groups based on the length of the Cys-rich region (Fig. 3) (Roy Choudhury et al. 2011; Mohanasundaram et al. 2022).

The role of type III Gγ proteins in controlling important agronomic traits in plants

The first reports of the role of type III Gγ proteins in the regulation of key agronomic traits are from the early 2000s, when the proteins were in fact not designated as Gγ proteins. Identification of two quantitative trait loci (QTL), *GS3* (*Grain Size 3)* and *DEP1* (*Dense and Erect Panicle 1*) for controlling grain length and panicle erectness, respectively, in rice, and subsequent cloning of the underlying genes was the first indication of the role of these proteins in regulation of rice yield (Fan et al. 2006, 2009; Huang et al. 2009; Takano-Kai et al. 2009; Mao et al. 2010). Later, the AGG3 protein was identified in Arabidopsis (Chakravorty et al. 2011), and the SGG8-10 proteins were identified in soybeans (Roy Choudhury et al. 2011) by homology-based searches with canonical Gγ proteins and their obligatory interaction with the Gβ proteins. The existence of a Cys-rich region with the canonical Gγ-like domain led to their classification as plant-specific, Gγ proteins. Only after their characterization in Arabidopsis and soybean proteins as unique variants of Gγ proteins in plants, GS3 and DEP1 were classified as a part of the plant G-protein complex. In Arabidopsis, AGG3 was predominantly characterized for its role in the regulation of abscisic acid (ABA)-dependent abiotic stress responses (Chakravorty et al. 2011; Thung et al. 2012). The *agg3* knockout mutants of Arabidopsis are hypersensitive to ABA for germination and are hypersensitive to several abiotic stresses (Chakravorty et al. 2011). Overexpression of the *AGG3* gene in Arabidopsis and *Camelina sativa*, an oil seed crop of Brassicaceae family, resulted in better tolerance to exogenous ABA, high salt, and heavy metals (Roy Choudhury et al. 2014). An independent study in Arabidopsis also identified *AGG3* as an organ size regulator, by map-based cloning. The *agg3* knockout mutants have slightly smaller leaves and flowers (Li et al. 2012a, 2012b). These data were confirmed by overexpression of the *AGG3* gene in *Camelina sativa,* which resulted in larger reproductive organs and larger seeds, ultimately leading to higher oil yields (Roy Choudhury et al. 2014). These observations implied that the type III Gγ proteins control two different aspects of plant life: (i) the overall development, especially the development of reproductive organs; and (ii) the response to abiotic stresses. Studies in other crops, especially rice, have corroborated the dual roles of type III Gγ proteins.

The role of type III Gγ proteins in the regulation of cereal seed traits

Rice *GS3* was the first gene cloned for grain size regulation (Fan et al. 2006, 2009) and *GS3* and *DEP1* remain some of the most extensively researched genes for their biotechnological applications. A quick survey of rice literature reveals more than 100 publications, ranging from the discovery of these genes as major QTL for several agronomic traits (Fan et al. 2006; Huang et al. 2009; Yu et al. 2011; Lu et al. 2013; Xu et al. 2015; Deng et al. 2022; Malik et al. 2022), their application in breeding (Wang et al. 2011, 2022; Yu et al. 2011; Xu et al. 2016, 2019; Nan et al. 2018; Rasheed et al. 2022), an artificial positive selection of specific alleles in domesticated varieties (Mao et al. 2010; Lu et al. 2013; Makino et al. 2020; Zhang et al. 2020), GWAS and haplotype analysis (Zhang et al. 2016; Zhao et al. 2016; Angira et al. 2022; Li et al. 2022; Wang et al. 2022; Zhou et al. 2017; Wu et al. 2020; Feng et al. 2016; Zaw et al. 2019; Malik et al. 2022), *RNAi*- and CRISPR-based gene editing (Li et al. 2016; Fu et al. 2019; Cui et al. 2020; Usman et al. 2021; Sun et al. 2018), and the overexpression of specific domains (Li et al. 2019; Zeng et al. 2019; Fan et al. 2009; Mao et al. 2010), to multiyear field trials (Wendt et al. 2016; Zhang et al. 2023). Specific alleles of *GS3* or *DEP1* are selected in modern cultivated crop varieties. For example, a favorable allele of *GS3* is highly enriched in a set of cultivated accessions (34%) compared to a set of wild accessions (4%) (Tao et al. 2017).

While the roles of GS3, DEP1 and GGC2, the third type III Gγ protein in rice in regulation of grain size is undisputed, whether the proteins are positive or negative regulators, and their mode of action are relatively unclear. For example, rice varieties carrying a wild-type *GS3* allele produce grains of normal length. Rice varieties carrying the complete loss-of-function allele eliminating the entire protein produce long grains, whereas rice varieties which express a truncated protein, *i.e.,* an intact GGL domain but no C-terminal region, produce very short grains (Huang et al. 2009; Mao et al. 2010; Botella 2012). In some instances, similar mutations have been reported to produce contrasting (short grain versus long grain) phenotypes. Analogous situations have been observed with the *DEP1* gene*,* which was identified as a key determinant of panicle architecture (Huang et al. 2009; Botella 2012). Specific substitutions in the *DEP1* gene resulted in either erect panicles with more branches and seeds or smaller panicles with fewer branches and fewer seeds. The phenotypes appear to depend on whether mutations remove the entire protein or only the C-terminal region, leaving the GGL domain intact (Huang et al. 2009, 2022; Botella

2012; Zhao et al. 2016; Ngangkham et al. 2018; Li et al. 2019). *GGC2*, the third type III Gγ homolog in rice was not identified in any GWAS or haplotype analysis, but designed mutations in this gene also resulted in altered plant architecture, including changes in panicle and seed morphology. *GGC2* has been proposed to have overlapping functions with *DEP1* (Sun et al. 2018; Chaya et al. 2022).

The use of CRISPR-based gene editing in recent years has offered some clarity to these confounding observations. Several groups have now created mutants expressing distinctive alleles of *GS3*, *DEP1,* and *GGC2* in rice (*e.g.,* full length versus C-terminal deleted versions) individually or in different combinations, to uncover their redundant versus specific roles in grain size regulation (Li et al. 2016; Cui et al. 2020; Usman et al. 2021; Sun et al. 2018). One such comprehensive study proposes that GS3, DEP1, and GGC2 act in an interrelated signaling module, together with the Gα (RGA1) and Gβ (RGB1) proteins of the G-protein complex to regulate grain size in rice. In this model, the grain size is proposed to be directly regulated by DEP1 and GGC2, but not GS3. Instead, GS3 negatively regulates DEP1 and GGC2 (Sun et al. 2018) (Fig. 4). While this model may explain the phenotypes in a specific context, *i.e.,* grain size, it is far from complete. It offers no explanation for the roles of other proteins of the complex such as the two canonical Gγ, RGG1 and RGG2, which also form obligate dimers with the same RGB1 protein, or how might the four additional Gα proteins (XLGα 1–4) be involved. The model also does not conform to the classical G-protein signaling module, as it places RGA1 downstream of RGB1 and upstream of DEP1 and GGC2. It is plausible that the canonical G-protein signaling model is not followed during the regulation of grain size, but its ubiquitousness during the regulation of other phenotypes by the same genes remain unknown.

The contrasting effects seen upon full length versus C-terminal deletion have been explained based on the modular nature of type III Gγ proteins (Botella 2012). The consensus in the field is that the unique Cys-rich C-terminal region of these proteins acts as a negative regulator of the GGL domain function. According to this model, a naturally occurring or engineered mutation at the beginning of the protein will remove the entire protein to generate a complete loss-of-function mutant. In contrast, a mutation that results in the deletion of the C-terminal region will still retain an intact GGL domain. In this case, the negative regulation by the C-terminal domain is alleviated, allowing the GGL domain to function. Consequently, C-terminal versus full-length deletions may result in opposite phenotypes (Fig. 5). Another less likely situation, where the GGL domain is lost, but the Cys-rich region remains may also exist and could have different effects than the complete loss of function mutant (Fig. 5). Although this model offers an explanation for several traits observed with the full length versus C-terminal deleted versions of the proteins, several questions remain. For example, is there an effect on protein stability when full length versus truncated protein is expressed? This is pertinent as some studies propose regulation of type III Gγ proteins via ubiquitin proteasome-mediated pathway (Yang et al. 2021). It is also not known how these specific alleles might differ in their interaction with Gβ, or influence the stoichiometry of Gβ versus five different Gγ interactions.

Incidentally, studies showing the effects of the overexpression of only N- or C-terminal regions of proteins in rice are inconsistent and an overall conceptual reconciliation of the models presented in Figs. 4 and 5 remains complicated. An additional question is related to the expansion of the C-terminal region in these proteins. There is also no explanation, to date, for why the C-terminal region is expanded in different proteins within a single plant species, and how the difference in its length could affect its role as an inhibitor of the GGL domain or the overall protein function. Moreover, at least in Arabidopsis, the entire protein is required for its function and expressing individual domains has no effect on plant phenotype (unpublished data).

A few studies in wheat, barley and maize have assessed the roles of the homologs of type III Gγ proteins with varying degrees of success. A 12-year field study in barley showed that the loss of function of *HvDEP1* resulted in consistent effects on stem elongation and grain size but conferred either a significant increase or decrease in harvestable yield depending on the environment (Wendt et al. 2016). In wheat, a survey of *DEP1* gene sequence in species with normal, compactoid and compact spikes did not

Fig. 4 Proposed model for type III Gγ protein-regulated grain size control in rice (adapted from Sun et al. 2018)

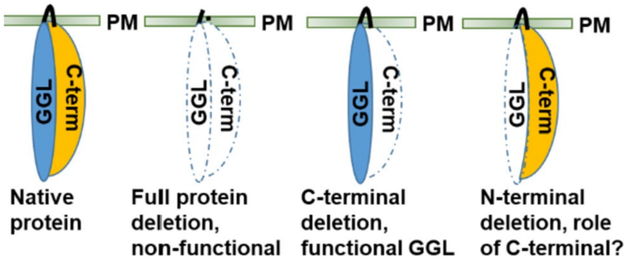

Fig. 5 A simple cartoon to explain distinct phenotypes observed by removing specific domains of type III Gγ proteins. PM- plasma membrane

identify any specific changes that correlated with the phenotype (Vavilova et al. 2017; Yang et al. 2019). Some association has been seen in kernel size and *GS3* gene variation in maize, but unlike rice, the gene does not appear to be under any positive selection (Li et al. 2010). Recent data on the roles of type III Gγ genes in sorghum suggests their involvement in regulating key developmental processes. One study has identified *SbDEP1* (SORBI 3002G216600) as a possible locus responsible for grain size differences between different landraces of sorghum (Tao et al. 2017; Zou et al. 2020). *SbGS3* homolog (SORBI 300G341700) has been identified as the gene underlying the QTL *qTGW1a*, which is a negative regulator of seed size (Zou et al. 2020). The same locus has been identified as the causal gene that controls the glume coverage in sorghum seeds and is named *Glume Coverage 1* (*GC1*) (Xie et al. 2022). Analysis of 915 diverse accessions of sorghum for glume coverage and its relationship to domestication identified *GC1* as the main effect and stable locus for this trait. Sequence analysis of 482 sorghum accession identified alterations in the fifth exon of the gene, causing deletion of the C-terminal region (similar to rice *GS3*), which showed a strong association with glume coverage. Transgenic expression of truncated versions of *GC1* (*SbGS3,* SORBI 300G341700) in sorghum resulted in seeds with reduced glume coverage. Surprisingly, this study did not identify any strong association between these variations and grain size (Xie et al. 2022).

In summary, these data suggest that the type III Gγ proteins are involved in the regulation of grain size, number, or development. However, carefully designed, targeted studies to evaluate the roles of individual domains in the context of G-protein complex are needed to uncover their mode of action. Furthermore, as is evident from the barley field trial (Wendt et al. 2016), multiyear field trials of plants expressing specific alleles in pertinent species are necessary to realize their full agronomic potential.

The role of type III Gγ proteins in regulating abiotic stress responses in cereals

As mentioned earlier, the type III Gγ protein in Arabidopsis was identified primarily for its role in abiotic stress tolerance (Chakravorty et al. 2011; Roy Choudhury et al. 2014). The effect of these proteins in imparting stress tolerance seems to be more straight forward, compared to the regulation of seed size or plant architecture. In Arabidopsis and Camelina, overexpression of *AGG3* gene resulted in improved tolerance to various abiotic stresses. Targeted overexpression of a monocot codon optimized *AGG3* gene in *Setaria viridis* also led to improved tolerance to ABA or high salinity in plate-based assays, and to low nitrogen levels in adult plants (Kaur et al. 2018). Furthermore, Setaria plants overexpressing *AGG3* showed significantly improved tolerance to heat stress (HS) (Torres Rodríguez et al. 2023). The exposure of plants to a short duration HS (*e.g.,* 24 h) at the panicle emergence stage had a significant effect on plant survival and productivity at the later stages (Torres Rodríguez et al. 2023).

Two major recent findings in rice and other cereals have further solidified the roles of type III Gγ proteins in conferring stress tolerance to crop plants. Rice *GS3* has recently been identified as the causal gene underlying a QTL for heat stress tolerance and is named *Thermotolerance 2* or *TT2* (Kan et al. 2022). In contrast to the results obtained in Setaria, where the entire protein was needed for imparting stress tolerance, the effects of TT2 seem to be dependent on the presence of a specific domain of the protein. Plants carrying the functional *TT2* allele exhibited sensitivity to heat stress, whereas those with a natural mutation that disrupts the full-length protein and removes the C-terminal region were significantly tolerant to heat stress. The enhanced thermotolerance of these plants was due to a reduction in the wax content compared to plants that carried the functional *TT2* allele.

Additionally, the *GS3* gene in sorghum has been identified as the underlying QTL for alkalinity stress tolerance and named *Alkaline Tolerance 1* (*AT1*) (Zhang et al. 2023). Similar to *TT2*, the mutations causing the C-terminal truncation (*at1* allele) increased sensitivity, while complete knockout of *AT1* increased tolerance, to alkaline stress. These results were replicated in multiple field trials and in other crops such as rice, maize, and Setaria (Zhang et al. 2023).

A further role of these proteins has been described in influencing nitrogen use efficiency (NUE). Rice *GS3* has been implicated in improving NUE in the long grain *japonica* varieties (Li et al. 2022; Yoon et al. 2022), whereas *DEP1* has been identified as a major QTL for NUE (Sun et al. 2014; Xu et al. 2016, 2019) based on genetic analysis, but the underlying mechanisms of how these proteins affect NUE remains unknown. Several studies have also identified the roles of these proteins in drought tolerance (Cui et al. 2020; Majumdar et al. 2023) and in heavy metal stress tolerance (Kunihiro et al. 2013), but these have not been assessed at the level of field trials.

Surprisingly, the signaling mechanisms of type III Gγ proteins operative during regulation of these unique developmental transitions or stress responses remain varied. As key constituents of the plant heterotrimeric G-protein complex, type III Gγ proteins are expected to function with their obligatory partners, the Gβ proteins, which seems to be the case with Arabidopsis and Camelina mutants. The proteins also seem to have a major effect on the cellular redox status, which is not surprising, given the presence of an extremely high amount of Cys residues in their C-terminal region (Alvarez et al. 2015). But apart from this, attempts to identify signaling modules and downstream effectors of these proteins have led to disparate mechanisms, ranging from hormone signaling pathways, Ca^{2+}/CaM-dependent pathways, MAP kinases, lipid signaling, ubiquitin proteasome-mediated inhibition, interaction with several transcription factors and more (Jiang et al. 2018; Liu et al. 2018; Yuan et al. 2019; Zhang et al. 2019, 2023; Zhao et al. 2019; Chen et al. 2021; Wu et al. 2023; Roy Choudhury et al. 2014; Chakravorty et al. 2011; Wang et al. 2017; Kaur et al. 2018; Xia et al. 2018; Yang et al. 2021; Kan et al. 2022; Liang et al. 2022; Torres Rodríguez et al. 2023), as summarized in Table 1. These observations suggest that a comprehensive mechanism of action of how these unique proteins function remains to be established.

Conclusions, unanswered questions, and future perspectives

The above discussion highlights the agronomic potential of proteins such as type III Gγ proteins for improved yield and tolerance to abiotic stresses. Several studies in multiple plants, including currently cultivated crop species, confirm that altered expression of type III Gγ proteins will have important implications. It is noteworthy that there is a propensity for C-terminal deletions in many cultivated species (e.g., rice, sorghum Tao et al. 2017; Xie et al. 2022), suggesting that the C-terminal region of type III Gγ proteins is crucial for their function and may have been adapted for functions pertinent to specific species, that is, grain length in rice or glume coverage in sorghum. However, it is also obvious that several knowledge gaps remain that need to be addressed to ensure the full utilization of these proteins for future agriculture. The specific questions resulting from the previous research relate to both the characteristics of the protein itself and its function. For example, it is not known what is the role of GGL domain versus Cys-rich domain in regulation of specific traits? Are there regions within these domains that specify a function? How does deletion of specific domains by natural or created mutations affect protein stability and is there a difference in interaction strength or abilities of individual domains versus full proteins and does that influence the elicited response? What are the effects of eliminating one Gγ protein to the stoichiometry of Gβγ complex formation in planta? It is also surprising that most plants possess more than one member of this protein family, and it is not known why there is an extension in the length of Cys-rich region between them and what the implications are of short versus long cys-rich regions on protein function? Additionally, at the functional level it is not known if these proteins always work as a part of the trimeric G-protein complex or also have independent roles. Furthermore, while their roles have been identified in a range of different growth, development, and stress responses, it is not known how these are interconnected at the regulatory level. For example, the *GS3* locus in rice is reported to control grain length (*GS3*), alkalinity tolerance (AT1), and thermotolerance (*TT2*), with little overlap between their suggested regulatory pathways. Additionally, while each of these traits has been evaluated

Table 1 Proposed signaling modules involved in regulation of plant phenotypes via type III Gγ proteins

Species	Protein	Phenotypes	Proposed signaling modules	References
Arabidopsis	AGG3	Abiotic stress tolerance	ABA signaling, ROS-mediated pathways	Chakravorty et al. (2011), Roy Choudhury et al. (2014)
Camelina	AGG3	Abiotic stress tolerance	ABA signaling, ROS-mediated pathways	Roy Choudhury et al. (2014), Alvarez et al. (2015)
Setaria	AGG3	Abiotic stress tolerance	ABA signaling, ROS-mediated pathways, improved photosynthetic parameters	Torres Rodríguez et al. (2023), Kaur et al. (2018)
Rice	GS3 and DEP1	Grain size	MADS domain transcription factors OsMADS1	Liu et al. (2018)
Rice	DEP1 NIL-GS3	Grain size	Auxin, cytokinin and ABA content, starch biosynthesis genes	Zhang et al. (2019), Liang et al. (2022)
Rice	DEP1	Grain yield	TTP (TON1-TRM-PP2A) complex	Wu et al. (2023)
Rice	DEP1	Grain yield	C:N ratio, GS GOGAT pathway, RuBP and PEPC enzymes	Zhao et al. (2019)
Rice	DEP1	Grain yield	OsSPL18 regulation	Wang et al. (2017), Yuan et al. (2019)
Rice	DEP1	Grain size	microRNA 164b, OsNAC2	Jiang et al. (2018)
Rice	GS3	Grain size	Ring E3 Ligase dependent degradation	Yang et al. (2021)
Rice	GS3	Grain size	Interaction with Shaggy-like Kinase	Xia et al. (2018)
Rice	TT2 (GS3)	Thermotolerance	Ca^{2+}/CaM-dependent SCT1 regulation and wax biosynthesis	Kan et al. (2022)
Sorghum	AT1	Alkaline stress tolerance	Phosphorylation of aquaporins, H_2O_2 levels	Zhang et al. (2023)

in multiple field trials, the three variables have not been tested together. Because the same genes or substitutions do not seem to be under a similar positive selection in other plants such as maize, wheat and sorghum, their plant-specific functions, and the mechanisms behind them also need to be determined. A long term (12-year study) of HvDEP1 effects in barley found significant variation in year-to-year yield, ranging from lower than normal to higher than normal, depending on the environmental conditions (Wendt et al. 2016), emphasizing evaluations of key agronomic traits governed by these proteins in long-term field trials. And finally, as is seen for the myriads of downstream processes that are affected by them (Table 1), a better understanding of their regulators and effectors is also needed to realize their full potential.

Acknowledgements The author thanks current and former lab members for engaging discussion on G-protein signaling in plants.

Author contribution SP conceived and wrote the manuscript.

Funding Research in the Pandey lab is supported by grants from the National Science Foundation (MCB-1714693 and MCB-2207012) to S.P.

Declarations

Conflict of interest The author has no relevant financial or non-financial interests to disclose.

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