Characterization of the Heterotrimeric G Protein Gene Families in *Triticum aestivum* and the Caleosins *CLO3* and *CLO7* in *Brachypodium distachyon*

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Characterization of the Heterotrimeric G Protein Gene Families in *Triticum aestivum* and the Caleosins *CLO3* and *CLO7* in *Brachypodium distachyon*.

Abstract

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In the last few decades global food security has been an important issue due to continuously growing population. The common bread wheat, *Triticum aestivum,* belongs to the tribe Triticeae and serves globally as one of the most important staple foods. The minimization of the losses in the crop yield caused by biotic and abiotic factors will be a beneficial approach to increase the crop production. The identification of the genes that change gene expression in response to stresses has been an important avenue to identify candidate genes that may contribute to stress tolerance. Whole genome and transcriptomic analysis has accelerated the discovery and characterization of genes related to stress responses and tolerance. Several gene families in *T. aestivum* that respond to abiotic stress conditions have been identified and their characterization is in progress. Heterotrimeric G protein gene families have been long known to be involved in the regulation of plant growth and development under control and stress conditions and these gene family members are also found to play regulatory roles through interaction with other proteins. Here we have characterised the heterotrimeric G protein gene families in the *T. aestivum*. The heterotrimeric G protein α subunit (Gα) has been shown to interact with caleosins, a class of calcium binding proteins, and regulate stress responses through abscisic acid signalling. *T. aestivum* Gα is known to interact physically with *Caleosin 3*. However, the facility for genetic studies in *T. aestivum* is somewhat limited due to the lack of a readily available set of mutants, it's polyploid nature and difficulty for transformation. *Brachypodium distachyon* has been developed as a model experimental species for monocotyledons with the particular relevance to the crop species in the Triticeae. It is important to know if the caleosins in Brachypodium also interact with its Gα subunit and the interaction is conserved among the species. In addition, Brachypodium lines with mutations for two caleosin genes are available and in this study have been characterized for their effects on root growth in response to abiotic stresses.

The first study (Chapter 2) of this thesis characterises the heterotrimeric G protein gene families in *T. aestivum.* Two of the Gγ' were validated through *in vivo* protein-protein interaction by bimolecular fluorescence complementation. The differential expression analysis using RNA-Seq and microarray analysis showed that at least one homeologous gene copy of these members responded to abiotic stress conditions such as drought, heat, or cold, whereas only the *Gγ1* paralog was found to be induced after inoculation of spore suspension of the fungus *Fusarium graminearum* in the resistant line NIL38 compared to the *F. graminearum* susceptible line NIL 51. This study will create a rationale to elucidate the possible role of heterotrimeric G protein gene family members in wheat under these stress conditions, which can be further investigated through mutant analysis.

The second study (Chapter 3) of this thesis report the physical interaction of *B. distachyon* heterotrimeric G protein subunit Gα with its *CALEOSIN 7* (Bd-*CLO7*) and the role of *CLO7* in regulation of root growth. We investigated the effect of Brachypodium *CLO7* mutation on the regulation of primary, coleoptile node and lateral root growth under ABA and osmotic stress. Brachypodium *CLO7* has found to regulate the lateral root growth under osmotic stress through ABA independent signalling.

The third study (Chapter 4) of this thesis determines the physical interaction of Brachypodium *CALEOSIN 3* (Bd-CLO3) and, its N and C terminal truncations with Bd-Gα. We investigated the role of Brachypodium *Caleosin 3* in the regulation of primary, coleoptile node and lateral root growth under ABA and osmotic stress by mutant analysis. Bd-*CLO3* has been found to affect the primary root growth under ABA and osmotic stress and negatively regulate the coleoptile node root growth under non stress and osmotic stress conditions. In addition, Brachypodium *CLO3* negatively regulates the lateral root growth through ABA signalling, whereas under osmotic stress it affects lateral root growth through both ABA dependent and independent pathways.

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Contribution of Authors

I am the first author of all the chapters in thesis. I have conducted experiments collected, analysed the data, and wrote all the four chapters of the thesis. Dr. Gulick contributed in building the conceptual framework and revision of the chapters. Each chapter is prepared as manuscript for submission in the peer-reviewed journal.

Chapter 2

I am the first author of this manuscript. I have carried out the data collection, analysis and writing of the manuscript. Dr. Gulick developed the conceptual framework and helped in the revision of the manuscript. I have cloned the Gβ and Gγ's and studied their *in vivo* protein interaction using *Nicotiana benthamiana*. I collected RNA-Seq data for the tissues and under stress conditions from public data repositories and analysed all the sequences and individual gene expression profiles. Sabrina Brunetti helped with the RNA-Seq analysis.

Chapter 3

I am the first author of this manuscript. I made the constructs for the protein-protein interactions and carried out the *in vivo* experiments. I have standardized the protocols for Brachypodium root growth under ABA and mannitol stress conditions. I collected and analyzed the data. Dr. Gulick developed conceptual framework for the work and helped in the revision of the manuscript.

Chapter 4

I am the first author of this manuscript. I made the constructs to study the protein-protein interaction by BiFC and yeast two hybrid analysis. I conducted all the experiments, collected and analyzed the data and wrote manuscript. Dr. Gulick developed conceptual framework for the work and helped in the revision of the manuscript.

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Chapter 1: General Introduction

The continuously increasing world population has set an alarm for agriculture scientists to meet the demands of food by increasing the food production in the near future and the Food and Agriculture Organization of the United Nations (FAO) has predicted that the demand for the food will be doubled by 2050 (Godfray et al., 2010). Global food security has been prioritized even by United Nations and has been ranked second among the 17 agendas in 2030 Agenda for Sustainable Development (Berners-Lee et al., 2018). The major source of food in most of the parts of the world comes from wheat, rice and barley which belongs to family Poaceae and, among these crops, wheat is one of the important cereals crops cultivated and consumed globally. The increase in the agriculture produce of staple foods by the minimization of the heavy losses cause by environmental stress conditions is an important approach to increase to the crop production. The stress conditions can be categorised in two groups, one which involved damage caused by biological agents like pathogens and insects is categorised as biotic stress, and another category involves the stress caused by abiotic factors including water deficiency or excess, cold, salt, heat, toxic heavy metals and ultraviolet radiation. The effect these stresses on the plant depends upon the intensity, time period of stress, quantity and the mode of action of the stress. Often the abiotic stress factors create the favourable environment for the infestation of biotic factors like insect pests and pathogens (Ansari et al., 2019; Pandey et al., 2017). The major abiotic stress conditions in wheat include drought, extreme heat, salinity and cold stress. The increasing instances of drought and extreme heat stress due to changing climate conditions in wheat growing areas especially those having suboptimal conditions lead to the reduction in the yield and create the need to import wheat from other parts of the world (Enghiad et al., 2017).

Plants have their own mechanisms to cope with the environmental stresses. For example, the plants respond to drought stress through three different mechanisms that include escape, avoidance, and tolerance. However, it is not necessary that plant would develop only one of these mechanisms and a combination of more than one of these can also be used by plants to respond to drought stress (Levitt, 1972; Ludlow, 1989). A common escape mechanism is the development of a shortened lifecycle, and thus completion of the lifecycle before the onset of the drought conditions; it is associated with the successful reproduction before the occurrence of stress. These mechanisms are observed primarily in arid regions where the plants have short life cycle and high

growth and gaseous exchange rates. The avoidance mechanism in plants includes the adaptive responses associated with stomatal control, changes to light interception and canopy structure which help the plants to minimize water losses. Plants close their stomata, roll their leaves, and shed older leaves in the avoidance mechanism. The tolerance mechanisms involve the osmotic adjustment in the plants at cellular level, such as formation of rigid or small cells or accumulation of osmolytes (Chaves et al., 2003) which help plants to maintain the water status under drought stress.

The differential expression of genes under stress suggests their involvement and possibly their role in tolerance to the particular stress conditions. Genes are either upregulated or downregulated in response to the stresses and this can be measured by the use of high-throughput next generation sequencing such as RNA-Seq which has revolutionized transcriptomics and largely replaced hybridization based microarrays. The second generation sequencing technologies have opened a new channel in the gene expression profiling at different growth stages of the crop plant species and in response to the abiotic and biotic stress conditions in the globally important crops such as *T. aestivum* (Liu et al., 2015; Goyal et al., 2018; Boedi et al., 2016). The heterotrimeric G protein gene families in plants are known to be involved in different stress responses and the study of these gene family members in crops like wheat will be helpful in determining the role of each subunit in stress response. Plants have lower number of heterotrimeric G protein subunits compared to animals which indicates that single subunits can be involved in different stress responses. Hence, the second chapter of this thesis characterizes the heterotrimeric G protein gene families in *T. aestivum* and studies the gene expression for these gene family members in different plant tissues and in response to abiotic stress such as osmotic, heat, and cold stresses, and in response to pathogen *F. graminearum* using RNA-Seq 454 sequencing libraries and Affymetrix microarray analysis. The aim of this study is to identify the genes that are involved in regulation of plant growth and in stress responses which can be further studied using mutants. In the thesis, the terms “G protein” and “heterotrimeric G protein” are used as synonyms for each other which represent the heterotrimeric G proteins consisting of Gα, Gβ and Gγ subunits.

1.1. Heterotrimeric G protein signalling

In plants, the cell signalling is an important aspect in the regulation of the normal growth and

development, and responses to environmental cues. The primary signal for the stresses is generated by factors like antigens, hormone, light, neurotransmitters, odorants or another cell surfaces (Trewavas and Malho, 1997) which are then perceived by the membrane receptors and activate downstream effectors in the signalling pathway. In animals, heterotrimeric G protein complex consists of GDP bound Gα, and the Gβ and Gγ subunits, which is bound to the 7 transmembrane domain (7TM) G protein coupled receptor (GPCR) at resting stage of signal transduction (**Figure 1.1**). The signal perception or binding of ligands to G-protein Coupled Receptors (GPCR) triggers the exchange of GDP for GTP and activates Gα causing the conformational changes and the further release from the heterotrimeric complex and dissociation from Gβ-Gγ dimer.

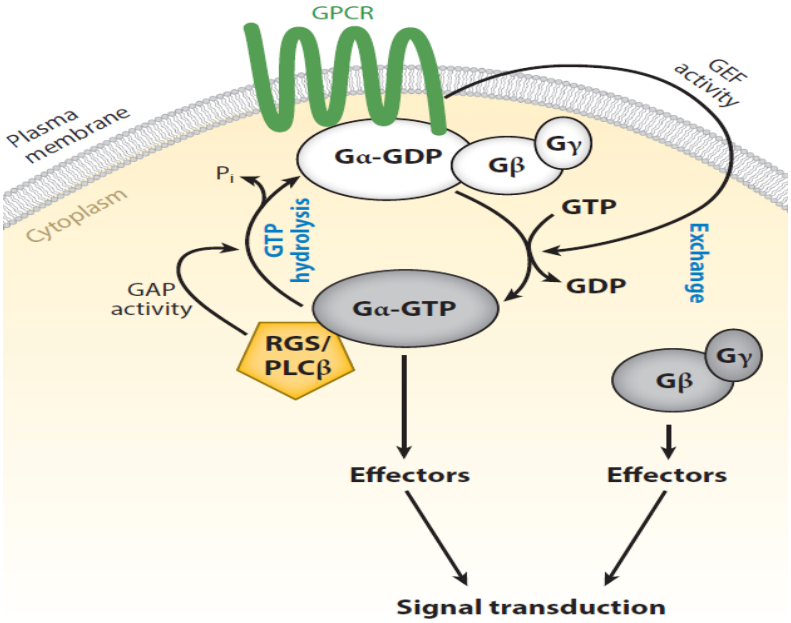

Figure 1.1. Conventional G protein signalling mechanism (Pandey, 2019)

The activated Gα-GTP and Gβ-Gγ dimer are ready to interact with the proteins called effectors (Urano and Jones, 2014). The conversion of GTP to GDP by hydrolysis permits the reconstitution of the trimetric complex. The plant Gα subunit is maintained in an active state due to the property of continuous exchange of GDP for GTP and in this case GTPase accelerating proteins known as Regulators of G protein Signalling (RGS) mediate the downstream signalling and returns Gα to its inactive GDP bound form by GTP hydrolysis. However, the mechanism of repression by RGS

in response to the different stimuli not known (Bender and Zipfel, 2018). Gα has three flexible regions called switch I, II and III which change confirmations in response to GTP binding and hydrolysis. The Gβ subunit is characterized by the presence of seven WD-40 repeats that form antiparallel β strands. These seven WD-40 repeats form the seven bladed propeller or torus like structure of Gβ, whereas N terminal region forms an α helix (McCudden et al., 2005). Gγ is folded into N-terminal and C-terminal α helices that form a structure by interaction with the α helix of Gβ and contact with Gβ torus respectively (McCudden et al., 2005). In addition to ligand binding activity, a GPCR acts as a Guanine nucleotide exchange factor (GEF) and facilitates the conversion of the Gα in the GDP bound state to the GTP bound state. The GTP hydrolysis of Gα which precedes the reconstitution of heterotrimeric complex is facilitated by proteins like RGS (Regulators of G protein signalling) which are called GTPase activity accelerating proteins (GAPs). Plants do not have GPCRs with GEF activity (Pandey, 2019). The active form of the Gα subunit in Arabidopsis, AtGPA1, is maintained through the spontaneous rapid nucleotide exchange rate in combination with the slower hydrolysis of GTP (Johnston et al., 2007).

The Arabidopsis genome encodes a single Gα subunit GPA1, a single β AGB1 and three Gγ's namely, AGG1, AGG2 and AGG3. This is in stark contrast to the multiplicity of G protein subunits in animal genomes; for example, the human genome encodes 23 Gα subunits, 6 Gβ subunits and 12 Gγ subunits. Though plant genomes encode fewer G protein subunits than animals, there is some variation for the number of G protein genes in different species, for example soybean, *Brassica rapa* and rice encode 10, 5 and 5 Gγ subunits respectively (Choudhury et al., 2011; Arya et al., 2014; Trusov et al., 2012). Heterotrimeric G proteins in plants have been known to be involved in seedling development, morphological development, cell proliferation, in the regulation of ion-channels and stomatal aperture and light perception. They are also reported to be involved in the hormonal signalling pathways that include abscisic acid, auxin, brassinosteroid, ethylene, gibberellins and jasmonic acid (Trusov and Botella, 2016). The expression studies in rice showed that genes encoding G proteins gene families were upregulated in response to abiotic stresses such as drought, cold and salinity (Yadav et al., 2012). This suggests that plants species possess variable numbers of heterotrimeric G protein subunits and these subunits are involved in different mechanisms including both plant developments and stress responses.

1.2. Bread wheat, *Triticum aestivum*- cereal of choice for many

The common bread wheat, *Triticum aestivum,* is the globally important staple food and it fulfills 20% of the mankind's protein and dietary caloric requirements. It was the first domesticated crop in the ancient civilizations like West Asia, Europe, and North Africa (Giraldo et al., 2019). The genome of the allohexaploid bread wheat, *Triticum aestivum*, (AABBDD, 2n=46), is comprised of three diploid genomes formed by the two polyploidization events. The first polyploidization event took place between the AA genome species *Triticum urartu* and a species related to *Aegilops speltoides* which contributed the BB genome around 0.5 million years ago which led to the formation of tetraploid AABB genome of *Triticum turgidum* ssp. Diccocoides. The second polyploidization event occur around 8,500 yrs. ago between the *T. turgidum* and DD genome species *Aegilops tauschii* and gave rise to todays bread wheat species *T. aestivum* with an AABBDD genome (Matsuoka, 2011). Three closely related homeologous genomes A, B and D share approximately 95% identity in the coding regions (Krasileva et al., 2013). It can be estimated that the high degree of similarity in the homeologs can be due to their functional redundancy, however varied expression patterns in the developmental as well as in response to stress has been found among the homeologs (Khalil et al., 2014). Interchromosomal rearrangements in the wheat genome had been occurred before the second polyploidization event. For example, the rearrangement between 4A, 5A and 7B chromosome in *T. aestivum*, was initiated with the 4A and 5A rearrangements, which are also reported in *T. urartu* and *T. monococcum*, occurred before the first polyploidization event. A second translocation between the 4A and 7B chromosome reported in the tetraploid wheat, occurred before the second polyploidization event. Other chromosomal rearrangements include reciprocal translocation between 5B and 7B in European wheat and translocation between 5B and 6B chromosomes in Ethiopian tetraploid wheat (Ma et al., 2015). Difference in the expression of homeologous genes, i.e. the same gene located in the different subgenomes, can be found in the allohexaploid wheat species and their parent species. These changes in the gene expression can be associated with cis-regulatory regions, epigenetic mechanisms, chromosomal rearrangements, deletion in the genome, epistatic and regulatory interactions in progenitor species (Chen and Ni, 2006). The wheat A homeolog for G protein alpha subunit in *T. aestivum* (Ta-Ga-7A) interacts with the protein COLD1, to regulate plant height, whereas the B copy of it does not interact with same protein due to the deletion in the C-terminal residues, showing that the specific homeologs may

be functional and interact with other proteins to regulate particular phenotype (Dong et al., 2019). This suggests that *T. aestivum* has a complex mechanism of gene expression and the varied expression patterns between homeologs can affect their contribution in the development of plant phenotype.

Bread wheat is affected by different environmental stress conditions including drought or osmotic stress that have different effects at different plant developmental stages including anthesis, germination, grain filling and tillering stages, that can reduce the crop yield. However, drought tolerance is also associated with the ploidy level; it was found that the hexaploid wheat are more drought tolerant than diploid and tetraploid cultivars (Abhinandan et al., 2018). Wheat can be grown at varying ranges of temperatures as high as 47.5 ± 0.5°C and as low as -17 ± 1.2°C (Porter and Gawith, 1999). The yield losses due to the heat stress is depend upon the developmental stage at which the crop is affected. High temperatures can damage the plants at the germination stage and can affect emergence. Other stages at which heat stress can cause yield losses are anthesis and the seed set stages (Abhinandan et al., 2018). Similarly the cold stress causes damage in the reproductive tissues of wheat that include gametophytes and pollen (Subedi et al., 1998) which leads to the losses in crop yield. Winter wheat is protected from the low temperature by delayed development of inflorescences, which only differentiate in the spring. Differential gene expression study in the wheat in response to abiotic stresses such as drought, heat and cold will give a preliminary idea about the genes involved or affected and will be useful to design the tolerant genotypes using genetic modifications.

1.3. *Brachypodium distachyon* a model for cereals

B. distachyon distachyon is a diploid (2n=20) species that belongs to tribe Brachypodieae which is the sister tribe of temperate grasses tribes Triticeae, Poeae, Bromeae and Avenae that include the important cereal crops like wheats (*Triticum* spp.) barley (*Hordeum vulgare*), rye (*Secale cereale*) and oats (*Avena sativa*). Brachypodium can be used as the representative of the temperate grasses to elucidate the gene functions. (Draper et al., 2001). Among *B. distachyon* accessions Bd21 and Bd21-3 diploid inbred lines has been commonly used and complete genome annotation for these species is available at Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html). The genome size of both these accessions is approximately 272 MB (International Brachypodium Initiative, 2010). Bd21 and Bd21-3 do not

differ from each other in terms of general appearance but they are different from 19 other accessions of Brachypodium (Vogel et al., 2006). The characteristic features like small stature, short life cycle, small genome size and simple growing habit make the Brachypodium a good choice for a model crops. The important agronomic traits of the temperate grasses are shared by Brachypodium and make it a good choice for the study of cereal genomics. Brachypodium has been estimated to be diverged from the *Triticeae* 32-39 million years ago, whereas the wheat and rice have a divergence period of more than 50 million years (Vogel et al., 2006). This places the Brachypodium closer to barley and wheat than monocot species like sorghum, rice and maize and hence makes it a better model to study the wheat functional genomics. Brachypodium is C3 plant like wheat, barley and rye, thus similar kinds of the adaptive responses to environmental cues can be expected in these species (Des Marais and Juenger, 2016). The similarity in genes for stress tolerance like freezing tolerance has been found in the wheat and Brachypodium. Karsai et al. 2005 suggested that the COR gene induction was necessary for the freezing tolerance of the facultative genotypes in barley without need of vernalization. The freezing tolerance study in plants evaluated after 28 days of cold acclimatization in the Brachypodium genotypes Bd2-3, Bd3-1, Bd21, Bd30-1, Bd1-1, Bd18-1 and Bd29-1 showed that the Brachypodium gene *COR413* can be used as a freezing tolerance marker gene which has also been reported in wheat (Colton-Gagnon et al., 2013). Brachypodium *C-repeat binding factor* (*CBF*) genes, *CBF1* and *CBF2* were known to be induced by drought and salinity, whereas the gene *CBF3* was induced by salinity stress. These genes were found to be regulated in ABA independent manner, similar to that previously reported in Arabidopsis. This indicates that the stress responses and the gene functions in the Arabidopsis and Brachypodium could be conserved. Brachypodium has been widely used as the model for cereal-pathogen interaction, including the stripe rust pathogen of wheat and rust pathogen of barley, *Puccinia striiformis* (Bettgenhaeuser et al., 2018). Similar studies were carried out in the wheat stem rust pathogen *Puccinia graminis* f. sp. Tritici (Della Coletta et al., 2019). The effect of the disruption of brassinosteroid hormonal signalling using *brassinosteroid-insensitive 1* (*BRI*) gene on the disease resistance has been studied in Brachypodium and the responses exhibited by Brachypodium in conferring pathogen resistance were found to be similar to those in barley; the mutants were resistant to necrotropic and heminecrotropic pathogens (Goddard et al., 2014). This suggests that the responses for the pathogen resistance mediated through brassinosteroid in barley and Brachypodium are conserved. Together these studies

suggest that Brachypodium can serve as a promising model to study the stress responses in tribe Triticeae.

1.3.1. Brachypodium vs Arabidopsis root system comparison

The root systems in Brachypodium and Arabidopsis differ in few aspects (**Figure 1.2**). Both Brachypodium and Arabidopsis have single primary root, similar to other crop species like rice and corn, whereas wheat has three to five primary roots. The lateral roots are similar in both Brachypodium and Arabidopsis.

a) Arabidopsis root system b) Brachypodium root system

Figure 1.2. Root system Arabidopsis vs Brachypodium. The figures show the a) Arabidopsis root system of a 10 day old plant and b) Brachypodium root system of a 30 day old plant (Pacheco-Villalobos and Hardtke, 2012). PR, CR and LR denote the primary, coleoptile node and lateral roots respectively.

However, Brachypodium shows the multiple coleoptile node axile roots (CNRs) and leaf node axile roots (LNRs) emerge at the later stages, between the three leaf stage and the grain development stage (**Figure 1.3**). Generally two coleoptile node roots (CNRs) emerge post embryonically in Brachypodium, however nutrient availability determines the emergence of the stem node roots. Brachypodium and wheat do not have any differences in the root system at

anatomical level (Chochois et al., 2015). Moreover, Brachypodium is a C3 plant like wheat and can serve as a good model for wheat. The root system architecture of a plant is important for the uptake of unevenly distributed water and nutrients from the soil layers, hence it has vital role in coping with the abiotic stresses such as drought, salinity, waterlogging and nutrient deficiency through adaptation of the root system (Koevoets et al., 2016). However, the relationship between these factors is complex and involves interaction between different hormonal signalling pathways. The hormones are essential for the regulation of root growth under normal as well as stress conditions. For example, auxin is essential for the lateral root formation and is required for development at specific stages.

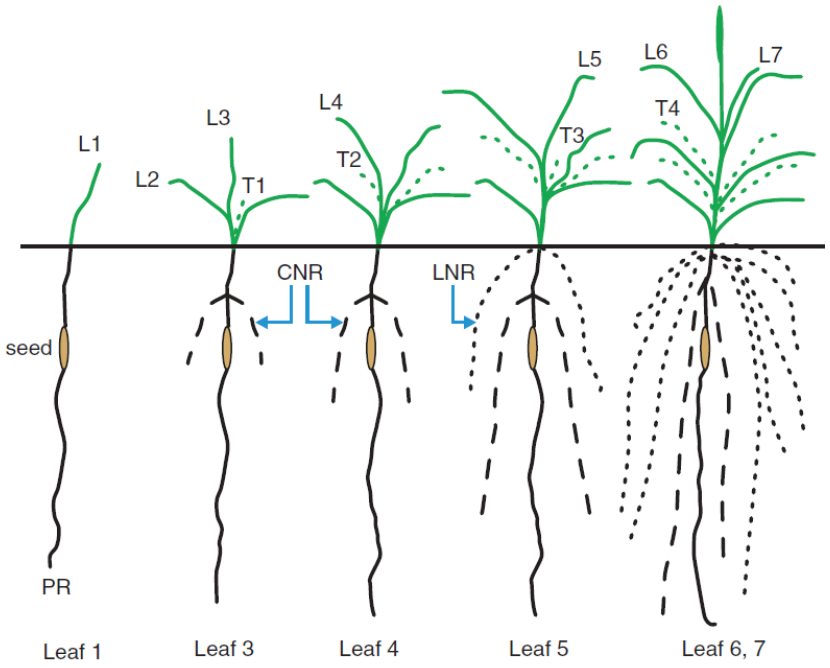

Figure 1.3. Coleoptile node root emergence at different stages. L and T are the leaf and tiller development stages respectively (Watt et al., 2009).

Defects in the lateral root formation had been found in mutants for indole-3-acetic acid inducible genes, *iaa14, iaa3*, *iaa19*, *iaa1*, *iaa18*, and *iaa28* which are known to be associated with auxin signalling pathways (Overvoorde et al., 2010). Auxin response factor such as ARF7 and ARF 19 are known to interact with these AUX/IAA proteins and *arf7/arf19* double mutants had the delayed lateral root formation (Okushima et al., 2005). Drought and salinity stress are associated with the increased level of ABA, which has inhibitory effect on the lateral root formation in the

post emergence stage (De Smet et al., 2003). Abscisic acid has an inhibitory effect on the root development when used at higher concentrations with the strongest effects seen in the inhibition of lateral roots development. However, the plant root system is also affected by the nutrients availability. For example, the study of lateral root initiation mutant (*lin1*) in Arabidopsis which carries mutation for nitrate sensor *NTR2.1* gene showed that nitrate has inhibitory effect on the LR elongation (Little et al., 2005). The concentration of 1 mM nitrate or phosphate had shown to inhibit the LR elongation in Arabidopsis, whereas the primary root growth was inhibited by phosphate (Linkohr et al., 2002). This indicates that the root system of plant is sensitive to the different hormones, nutrient and stress conditions, and the interaction of these factors can affect the root growth.

1.4. Heterotrimeric G proteins in regulation of root growth

The mutant analysis studies have shown that heterotrimeric G proteins in Arabidopsis are involved in the regulation of root growth under control as well as stress conditions. Arabidopsis AGB1 is the negative regulator of primary root growth under normal growth condition, whereas GPA1 shows wild type like primary root growth. The mutants *gpa1* and *agb1* have exactly opposite phenotype for lateral root growth and shows fewer and more lateral root growth compared to wild type, respectively (Chen et al., 2006). The similar reduction in the root growth for rice and maize Gα mutants *d1* and *ct2* had been found where mutants had 10% shorter roots and a 15% reduction seminal or crown root compared to wild types. Out of the three Gγ's in Arabidopsis AGG1 and AGG2 are involved in regulation of lateral root growth where both act as negative regulators of lateral root growth. Double mutant of *agg1/agg2* mimics the phenotype of *agb1* and produce more lateral root compared to *agg1* or *agg2* single mutants (Urano et al., 20016). Single or double mutants of *gpa1* or *agb1* in Arabidopsis were hypersensitive to the primary root growth inhibition when seeds were germinated for 24 hrs and were transferred on media supplemented with 2µM ABA. The wild type had 35% reduction in the root growth whereas *gpa1*, *agb1* or *gpa1/agb1* showed reductions of 55% and 80-85% respectively (Pandey et al., 2006). Altogether these results suggest that heterotrimeric G protein gene families in Arabidopsis are involved in the regulation of root growth under normal growth conditions and in response to ABA stress. Moreover, there is functional redundancy in regulation of root growth for these gene family members.

1.5. Caleosins are one of the GPA1 interacting proteins

Our lab has reported the *in vivo* interaction between Arabidopsis GPA1 and Clo3 and between GPA1 and Clo7 using bimolecular fluorescence complementation (BiFC) analysis (Wang, 2009). In addition, the Gα subunit from *T. aestivum* (GA3) has been reported to interact with the caleosin *Clo3* and phosphoinositide-specific phospholipase C (PI-PLC1), *in vitro* by pull down assays, and *in vivo* using BiFC in our lab (Khalil et al., 2011). The mutant analysis of the Arabidopsis *clo3* (*rd20*) showed that it has a role in ABA mediated seed germination, seed dormancy, stomatal control, transpiration and drought tolerance (Blée et al., 2014; Aubert et al., 2010). The *rd20* mutant had faster germination under ABA treatment conditions than the WT, whereas the overexpressor lines for *RD20* (*RD20-OE1* and *OE-2*) had lower and slower rates of germination in presence of ABA (Blée et al., 2014). In another study, the *rd20* mutant had higher water loss associated with increased stomatal opening and the mutant was more drought sensitive than the wild type (Aubert et al., 2010). The expression analysis showed 150 and 300 fold increase in the gene expression level of *RD20* after 2 hr of drought stress and 3hr of ABA treatment respectively, whereas the significant decrease in expression level for *RD20* was observed in ABA-deficient (*aba1-5*) or ABA-insensitive (*abi1-1*) mutants, showing that *RD20* is involved in ABA signalling. Moreover, *RD20* was shown to be expressed in the guard cells by *RD20::GUS* reporter gene (Aubert et al., 2010). In another study of Arabidopsis *Caleosin 4* (At-*CLO4*), the mutant At-*clo4* had the lower seed germination rate, whereas the overexpressor line for *At-CLO4* (AtCLO4-OX) had higher seed germination compared to wild types in the presence of ABA, clarifying that *CLO4* acts as a negative regulator in response to ABA stress (Kim et al., 2011). The interaction of the caleosins with GPA1 and their role in the regulation of stress responses through ABA signalling showed the possibility of link of G protein and ABA signalling pathway.

1.5.1. Calcium binding protein caleosins and their structure

The calcium binding proteins caleosins are found in the fungal species, algae and higher plant species, however they are not present in protozoans and animals (Partridge and Murphy, 2009; Hanano et al., 2018; Rahman et al., 2018). The Arabidopsis genome encodes seven caleosins, whereas in rice five caleosins have been identified. The bread wheat, *Triticum aestivum* and *Brachypodium distachyon* have eleven and ten caleosins per haploid genome respectively (Khalil

et al., 2014). The presence of hydrophilic N terminal (1-100 aa residues) and C terminal domains (137-245 aa), and a central hydrophobic domain (101-136 aa), are the primary components of the secondary structure model of sesame caleosin **(Figure 1.4).**

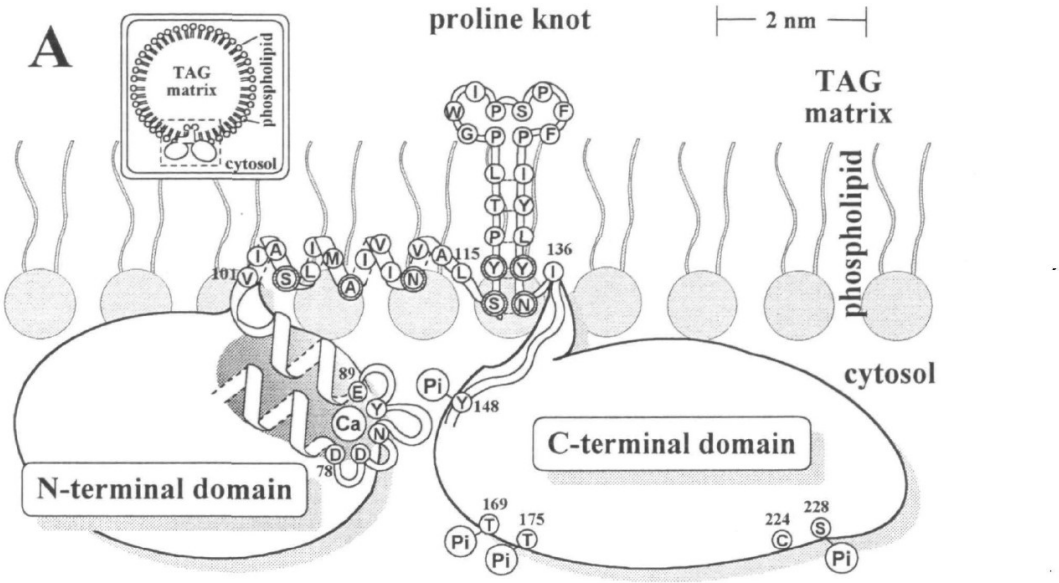

Figure 1.4. Structure of caleosins (Chen et al., 1999)

Both N- and C-terminal domains of caleosins are exposed to cytosol. The N-terminal domain is characterised by presence of a single EF hand calcium binding domain, the C-terminal domain has single tyrosine kinase and three casein kinase II phosphorylation sites. The central domain consists of the amphipathic α helix and proline knot like region (Chen et al., 1999). The proline knot is a proline rich region that is proposed to determine the conformation of the central domain. The EF hand motif in caleosin is the feature possessed by calcium binding proteins, which enable them to bind Ca2+ ions when the changes in the cytoplasmic Ca^{2+} level rise in response to stress conditions (Ranty et al., 2006). Khalil et al. 2011 had reported that the interaction between the *Triticum* GA3 and CLO3 proteins, tested by a His-tag pull down assay, was enhanced by the high Ca^{2+} concentration. The binding of GDP or GTP bound GA3 to the CLO3 had been found to be similar at the high Ca^{2+} concentration levels. This suggest that the presence of single EF hand calcium binding domain acts in receiving the environmental clues and that Ca^{2+} is an important factor in activation of Caleosins. The phosphorylation site at the C terminal ends suggests that

caleosins may be involved in the activation of downstream signalling cascade in response to the stresses.

1.6. Abscisic acid biosynthesis

The increase in ABA synthesis and its accumulation in the stressed tissues is a commonly observed phenomenon under drought stress condition. The ABA biosynthesis is controlled by the genes zeaxanthin oxidase (ZEP), 9-cis-epoxycarotenoid dioxygenase (NCED), ABA-aldehyde oxidase (AAO) and molybdenum cofactor sulfurase (MCSU) which are known to be induced by stress. The first step in the ABA biosynthesis is the ZEP mediated epoxidation of xiaxanthin to vioxanthin, which is further converted to 9-cis-epoxycarotenoid. NCED genes are responsible for the oxidative cleavage of epoxy carotenoid 9-cis-neoxanthin to intermediate compound xanthoxin, which is then further converted to ABA in cytosol by ABA-aldehyde (Xiong and Zhu, 2003). Increased ABA concentrations have been known to regulate the root and shoot growth in plants. The lower concentrations of ABA are known to promote the root growth, while the higher concentrations are known to inhibit root growth (Ghassemian et al., 2000).

1.6.1. ABA signalling pathway

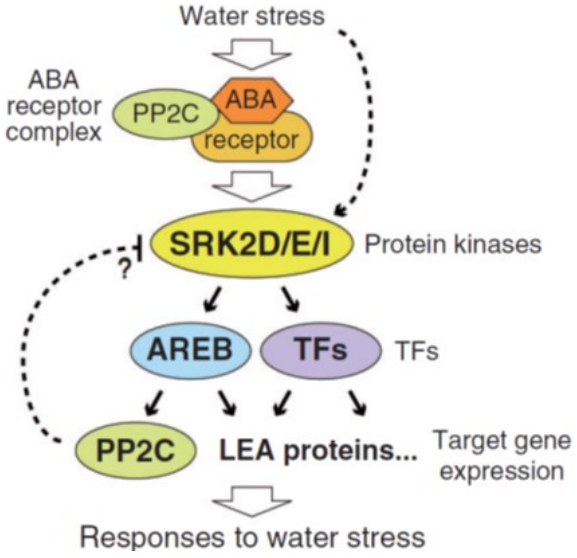

Figure 1.5. The core ABA signalling pathway in response to environmental stress

The core ABA signalling pathway includes ABA receptors, protein phosphatases and kinases **(Figure 1.5)**. The ABA receptors are encoded by a small gene family in plant species. The first ABA receptors were identified as pyrabactin resistance 1 (PYR1) and PYR1-like proteins (PYLs) which are also called regulatory components of ABA receptor (RCARs). In the presence of ABA, the ABA receptors PYR/PYL(PYR-Like)/RCAR interact and inhibit the activity of type 2 protein phosphatases (PP2Cs) and permits the SNF1-related protein kinase 2 (SnRK2) to be activated, which further phosphorylates downstream effectors including transcription factors known as ABA-responsive element binding factors (ABRE) (Fujii et al., 2009).

The mutants of the genes involved in ABA signalling as well as transgenic lines overexpressing these genes have altered responses to stress conditions. In Arabidopsis drought tolerance was exhibited by transgenic plants with the ABA receptor PYL9 under the promoter of an ABA induced gene, *pRD29A::PYL9.* The transgenic lines exhibited a reduction in transpiration rate and stomatal conductance; moreover the transgenic plants had enhanced water use efficiency and photosynthetic rates (Zhao et al., 2016). The overexpressor of the *Zea mays* PYLS, *ZmPYL8, ZmPYL9* and *ZmPYL12* had higher survival rates than wild type under drought stress showing that these genes have positive roles in regulation of drought stress (He et al., 2018). In rice, a member of the group A PP2Cs, *OsPP108* showed an increase in expression of more than 25 fold after 3 hr of incubation in 50 µM ABA (Singh et al., 2015). Drought stress of 1 hr and 3 hr induced the same gene more than 10-fold. The similar increase in expression of 15 fold was found after 6 hr of salt treatment. Transgenic lines overexpressing Os-PP108 had a higher seed germination rate under a 10 µM ABA treatment than the wild type, up to >80% vs. 25-30%. The similar responses were shown by the overexpressor in response to 175 mM NaCl and 375 mM mannitol; the transgenic line had a germination rate of more than 70%, whereas wild type had 45% and 60% germination in the treatments, respectively (Singh et al., 2015). In Arabidopsis, three SnRK2 (snrk2.2/2.3/2.6) genes out of the ten identified members of the SnRK2 gene family (SnRK2.1 to SnRK2.10) are known to be involved in ABA signalling. Futija et al. 2009 referred the triple mutant of Arabidopsis SNRK2 snrk2.2/2.3/2.6 as *srk2/d/e/i* which was found to be drought susceptible and died 7 days after the withdrawal of water irrigation and did not recover after re-watering in contrast to wild type. This triple mutant also had more water loss than the wild type. The differential expression analysis of *srk2/d/e/i* triple mutant and wild type in response 50 µM ABA and 250 mM mannitol or NaCl using microarray analysis showed the

significant changes in the expression of ABA and drought stress dependent genes. The marker genes involved in stress responses *KIN2, RD20, COR15A* and *RD29B* that are normally strongly up regulated by environmental stresses were no longer induced in the *srk2/d/e/i* mutant. The positive regulators in drought stress *RD26, DREB2A, AREB1* and drought stress responsive *LEA* genes did not show upregulation in *srk2/d/e/i* triple mutant when compared to wild type. These results suggest that the core ABA signalling pathway consisting of PYR/PYL (PYR-Like) /RCAR, PP2Cs and that three SNRK2's regulate ABA and stress signalling including response to drought and salt stress.

1.7. Root characters- an important trait in drought tolerance

The root system architecture of the plant is characterized by components including root length, root number, positioning and root component angle that determines the exploration of soil for water and nutrients (Koevoets et al., 2016). The roots are the first plant organ to sense the drought and transmit signal through cellular networks to generate adaptive responses thereby activating drought tolerance mechanism. The plant hormone abscisic acid has been known to act as a chemical messenger in the signal transduction. In order to maintain the plant water status, the plants reduce transpiration losses by stomatal regulation since the transpiration losses through the stomata accounts for 90% of the plants water uptake from the soil. Changes in transpiration are characterized by measuring stomatal conductance or stomatal aperture (Saradadevi et al., 2017). The maximum utilization of soil resources is related to the size of the plant roots system. The increased productivity under drought stress has been related to the different plant morphological characters which include total root length, root surface area, specific root length and specific root area of the plant (Comas et al., 2013). The higher yield in crop plants is associated with the deeper root system that efficiently utilizes the available nitrogen and water in the deeper soil layers. However, the balanced growth hypothesis suggest that under the moderate drought stress conditions, the plants water status is maintained through the reduction in shoot growth and maintenance or stimulation of root growth, which increase the root to shoot ratio of the plant (Bloom et al., 1985). The maximum water uptake from soil can be correlated with deeper root lengths which is associated with faster root system elongation and effective root angles. In wheat, a modelling study has suggested that the higher yield is associated with the narrow angle of the root penetration (Manschadi et al., 2007). This suggests that with the adaptation in the root

system, characters like root angles could also play a role in the acquisition of the soil resources. In rice breeding, the improved drought avoidance can be achieved by introduction of deep rooting trait in shallow root cultivars and the only a gene controlling the deep rooting QTL, *Dro1,* has been cloned in rice (Uga et al., 2011). Zhan et al. (2015) compared genotypes of two maize inbred lines, one with few but longer lateral roots (FL) and another with many but shorts (ML) lateral roots. Their results showed that the combination of reduced lateral root density and longer lateral roots had increased drought tolerance in maize. Under drought stress conditions, FL had significantly more relative water content and deeper roots than ML under greenhouse as well as field conditions. FL had a 144% higher grain yield than ML. However, the root characteristic that provide drought tolerance may differ between species and growth conditions, for example the reduction in the lateral root growth is common mechanism in response to drought, in contrast, the Arabidopsis mutant *enhanced drought tolerance1* (*edt1),* which has improved drought tolerance, has a primary root two times longer and more lateral roots than wild type, moreover the mutant has 30.5% reduction in the stomatal density (Yu et al., 2008). Another study on the maize root tip growth defective mutants *zmtip1-1* and *zmtip1-2* showed that these mutants had significantly shorter roots than the wild type. The overexpression of *ZmTIP1* in maize increased the root hair elongation and drought tolerance; the survival rate of 65% was found in these plants whereas wild type had survival rate of 35% under drought stress. This indicates that elongated root hairs make an important contribution to drought tolerance (Zhang et al., 2020). Altogether these results suggest that different root characters are associated with drought tolerance and longer lateral roots can be a positive component for drought tolerance.

1.7.1. Regulation of root growth: The interplay between ABA and auxin

The post embryonically formed lateral roots determine the maximum area of root system and are important in shaping the root system architecture of the plant (RSA) (Lynch, 1995). The primary roots are present as a radicle in the plant embryo (Grunewald et al., 2007). The lateral root growth can be divided into the initiation, lateral root primordium (LRP) development, emergence and meristem activation stages. However, the cells involved in the lateral root formation may differ from plant to plant. In dicot Arabidopsis, the lateral root is formed by the cell division in the pericycle, whereas in monocots maize and rice, LR is formed by cell division in the pericycle and endodermal regions. The lateral root initiation and formation of lateral root primordia (LRP)

is initiated by the increase in the levels of auxin in the basal meristem region. The auxin is accumulated in the xylem pole of the pericycle cells which act as founder cells for formation of lateral roots (Slovak et al., 2015). The auxin efflux carrier PIN3 maintains the reflux between the endodermis and pericycle, which is necessary for maintenance of auxin maxima for later stages in LR organogenesis (Marhavý et al., 2013). The transcriptional regulation in the LR formation is controlled by gene families of transcriptional regulators known as AUXIN RESPONSE FACTORs (ARFs) and AUXIN/INDOLE-3-ACETIC ACIDs (Aux/IAAs). SLR encodes a member of Aux/IAA gene family, IAA14 that affects the LR lateral root formation. The LR formation stage affected in the *solitary root mutant-1* (*slr-1*) was characterized by a cyclin-GUS chimeric protein, CycB1;1::GUS which detects the dividing cells in the pericycle and root apical meristem region during the lateral root initiation. The pericycle cells did not show CycB1;1::GUS activity in the five day old light grown *slr-1* seedlings; auxin induced initiation sites during the LR formation were found to be reduced in the mutant compared to wild type and CycB1;1::GUS activity was detected in the fewer cells of the older seedlings in the mutant than in the WT (Fukaki et al., 2002). In another study of double mutants of auxin response factors, *arf7 arf19,* mutants did not show expression of End199 marker line which acts in the stage II and later stages in the LRP development (Okushima et al., 2007). In maize, the AUX/IAA gene *Rootless with Undetectable Meristem 1* (*RUM1*) regulates the transcriptional activation of *Lateral Root Primordia 1* (*LRP1*). In the inbred line B73, *LRP1* was expressed in the lateral root and crown root primordia and the expression analysis in the five day old B73 primary roots showed that 5 µM concentration of 1-naphthaleneacetic acid 1 (1-NAA) induced *LRP1* more than three fold, after 3 hr of the treatments. This shows that *LRP1* in maize could have possible role in LRP formation through auxin signalling. This determines the complexity of the lateral root formation and the gene networks involved in shaping the fate of lateral roots associated with auxin signalling.

The hormone abscisic acid (ABA) is the key hormone in stress responses and it has also role in the plant development. In Arabidopsis, ABA is known to control the cell division and elongation stages in the root development. Its role in non stressed condition on the plant growth can be determined by the study of ABA deficient mutant *aba2/gin1* by Cheng et al., 2002, where the *aba2/gin1* mutant showed the phenotype of severe growth reduction in stems, siliques, roots, rosettes and cotyledons in the absence of exogenous sugar and stress. ABA regulates the root cell

division in primary root by inhibiting differentiation in the meristem region near the Quiescent Center (QC) and maintains the adjacent stem cell population, which are dividing cells, thus it regulates the dividing cells in the root tip (Harris, 2015). This suggests that ABA has an important role in the regulation of root growth in non stress conditions by controlling the QC region.

Besides its role in regulation of root growth under non stress conditions, ABA has inhibitory effect on the root growth under stress conditions. ABA is known to be induced by osmotic stress and it's role in the inhibition of the root growth under moderate osmotic stress conditions can be confirmed by the fact that the ABA biosynthesis inhibitor fluridon had rescued the root elongation under moderate osmotic stress (Rowe at al., 2016). The mutant analysis of ABA deficient mutant *abi1-1* and *aba2-1* had shown that the lateral root elongation is mediated through ABA dependent and independent pathways. Both the ABA and mannitol reduced lateral root elongation in *abi1-1* and *aba2-1* the mutants, however the degree of inhibition was different under these stress conditions. ABA is also known to be induced by salinity stress and to inhibit the root growth in Arabidopsis; however lateral roots are more sensitive than primary roots to the inhibition by ABA. In response to 1 μM ABA, the lateral roots were found to be more sensitive to the inhibition by ABA compared to the primary roots and showed the growth inhibition of 63% and 15.6% respectively (Duan et al., 2013). Similarly, Duan et al. 2013 showed that under salt stress lateral roots were more affected than the primary roots in multiple accessions of Arabidopsis. In response to 100 mM NaCl, the lateral and primary roots were inhibited by 84.3% and 49.6% respectively. Salinity stress affects the post emergence stages in the lateral root formation. These results together suggest that lateral root is more sensitive to repression by ABA than primary root and the lateral root growth inhibition under osmotic and salinity stress is mediated by ABA, though other ABA independent pathways are also involved.

1.8. Stress induced ABA dependent and ABA independent pathway

The characterization of stress responsive genes provides a view of the adaptive responses exhibited by plants under stress conditions. Drought and salinity stress are known to induce the stress responsive genes through ABA dependent and independent pathways (Yamaguchi-Shinozaki and Shinozaki, 2005). The induction of these genes requires the presence of cis-acting elements in the promoter regions to which various transcription factors bind and carry out their

transcriptional activation. The promoters regions of drought, salinity and cold stress induced genes are characterized by the presence of two cis- acting elements, ABA-Responsive Element (ABRE) and Dehydration-Responsive Element/C-RepeaT (DRE/CRT) (Yamaguchi-Shinozaki and Shinozaki, 1994). ABA-Responsive Element (ABRE) cis-acting elements function through ABA dependent pathway and is found in the promoter regions of ABA induced genes. For example, the ABA induced gene *RD29B* has two ABRE motifs ACGTGGC and TACGTGTC. The induction of *RD29B* under drought and salinity stress had also been confirmed by RNA gel blot analysis. *RD29B* was not transcriptionally induced in the ABA deficient mutant *aba1* by ABA, drought or salinity. This showed that the induction of *RD29B* under drought and salinity stress is ABA dependent (Uno et al., 2000). Altogether this indicates that cis-acting ABRE elements function in the ABA dependent gene expression under stress conditions.

DRE/CRT cis-acting elements are involved in the ABA independent gene expression under stress conditions. Drought stress inducible genes have DRE motif A/GCCGAC to which the Ethylene-Responsive element binding Factor/APETALA 2 (ERF/AP2) family transcription factors DREB1/CBF and DREB2 bind and carry out their transcriptional activation. For example, in Arabidopsis TFs DREB1A and DREB2A were shown to bind DRE motif in the promoter of the drought stress inducible gene *RD29A*. This *RD29A* has two DRE elements in the promoter region and the gene is transcriptionally activated through ABA independent signalling (Jia et al., 2012) in addition to ABA dependent signalling described above. The RNA gel blot analysis of dehydrated and salinity stressed 3-week-old unbolted Arabidopsis plants showed that *DREB2A* was highly induced within 10 minutes of dehydration or salinity stress, whereas the *DREB1A* gene had highest induction within 2 hr of cold treatments at 4°C. These results suggest that drought and salinity induces the transcriptional activation of *DREB2A* which further regulate the gene expression of *RD29A* (Liu et al., 1998). Sakuma et al. (2006) showed that transgenic overexpressor of Arabidopsis *DREBs, DREB1Ab* and the constitutive active form of DREB 2A, *DREB2A* CA, in which the residues between136 and 165 are deleted, were drought tolerant and had 60% and 62.8-83.3% survival rate when grown under water stressed for two weeks, whereas the wild type plants did not tolerate the stress and no plants survived. Among the 21 drought stress upregulated genes in *35S:DREB2A* CA transgenic plants 14 drought stress genes namely *RD29B*, *At1g52690*, *At1g69870*, *At3g53990*, *RD29A*, *RD17*, *LEA14*, *At2g23120*, *COR15A*, *KIN1*, *KIN2*, *COR15B*, *MT2A* and *At1g22985* showed the presence of DRE core motif

in their promoter regions. Similar responses had been found in rice where the overexpressor of Os-*DREB1* was tolerant to drought, salt and cold stress. In response to nine days of drought stress, the survival rate among independent transgenic lines after re-watering for 13 days for Os-*DREB1* overexpressor was found to be 17-80% whereas no wild type plants survived. Similar results were found for salinity stress imposed by 250 mM NaCl and cold stress imposed by 2°C, where the overexpressor had survival rate of 13-83% and 25-60% respectively. Most of the genes upregulated under drought stress also showed the presence of DRE core motif in their promoter regions (Ito et al., 2006). This suggests that the DRE core motif in the promoter region of genes is necessary for the induction of drought responsive genes, which are regulated by DREB TFs. The cis-acting elements other than DRE and ABRE had also been identified in the promoter region of stress inducible genes. For example, ABA and drought stress inducible gene *RD22* in Arabidopsis had two CACATG and single TGGTTAG recognition sites which are binding sites for AtMYB2 and AtMYC2 transcription factors, respectively (Abe et al., 1997). The gel blot analysis of *RD26*, which encodes a NAC transcription factor is induced by ABA, drought and salinity stress within 30 min of the treatments (Fujita et al., 2004). The ABA deficient mutant *aba2* had reduced *RD26* expression in response to dehydration stress, whereas salt treatment did not affects its expression. This indicates that the expression of *RD26* in drought is ABA dependent, whereas its response to salinity stress is ABA independent. The base substitution or deletion in the promoter region of RD29A gene in Arabidopsis had shown that ABRE functions in the ABA dependent gene expression under drought stress, whereas other cis-acting elements, including DRE, are also responsive to dehydration stress (Narusaka et al., 2003). Together these results suggest the complexity and involvement of the cis-acting elements in the promoter regions where ABRE and DRE are commonly found, however the cis-acting elements for TFs like MYB, MYC and NAC are also responsible induction of gene expression in response to stress.

With reference to this literature our study has two related parts, the first one is on the *Triticum aestivum* which is the second chapter of this thesis. The aim of this study is to characterize the heterotrimeric G protein gene families in bread wheat. We have identified the G protein gene family members and confirmed their characters with the protein-protein interaction using bimolecular fluorescence complementation. The differential expression in the developmental tissues and in response to stress condition is studied here. We have used RNA-Seq data in the public databases for gene expression analysis as well as available microarray data. This study will

give an insight in the possible role of heterotrimeric G protein gene family members in *Triticum aestivum* in different stress responses which can be further tested using mutants.

Another part of this thesis is the work on model plant for cereals, *Brachypodium distachyon*. Since wheat is hexaploid and Brachypodium is diploid, we choose Brachypodium for the functional characterization of the genes. Our lab has reported that in Arabidopsis At-*GPA1* interact with At-*CLO3/RD20* and At-*CLO7*. The current work expands this analysis to orthologs in Brachypodium which is third chapter of this thesis. The aim of this study was to determine whether CLO7 in Brachypodium interact with its Gα subunits and if yes how does the CLO7 regulate the root growth under normal, abscisic acid and osmotic stress conditions. This study also determines if the *CLO7* involved in the regulation of root growth through abscisic acid dependent or independent pathways.

Another work on Brachypodium of this thesis aim at determining if the interaction of CLO3 and Gα is conserved among plant species and if *CLO3* regulates root growth characteristics. Here we have tested protein-protein interaction for Bd-CLO3 and Bd-Gα. We studied the effect of the *clo3* mutation on the regulation of root growth under ABA and osmotic stress which will reveal the role of *CLO3* in the regulation of root growth through abscisic acid dependent or independent pathway.

Chapter 2: Characterization of the heterotrimeric G protein gene families in *Triticum aestivum*

2.1. Abstract

Heterotrimeric G protein gene families consist of Gα, Gβ, and Gγ subunits and genes encoding these subunits are diverse in numbers in different plant species. Genes encoding these members have been reported to be induced by biotic and abiotic environmental stresses and to regulate plant growth and physiological processes under normal as well as stress conditions by themselves or with other interacting protein partners. This study characterize the G protein gene families in *Triticum aestivum*, and their tissue specific expression patterns during course of development and in response to biotic and abiotic stress conditions. G protein gene families in *T. aestivum* are comprised of single *GA1*, single *Gβ* and four *Gγ* genes per haploid genome. Each member consist of three homeologous copies on A, B and D genomes except for Gγ3, of which B copy is translocated on A chromosome. G protein gene families were identified in monocot species such as *Aegilops tauschii, Brachypodium distachyon, Hordeum vulgare, Secale cereale, Setaria italica, Zea mays* and *Sorghum bicolor* and their evolutionary relationship were determined with *Oryza sativa* and dicot *Arabidopsis thaliana*. The tissue specific gene expression and altered gene expression in response to biotic and abiotic stresses for *T. aestivum* were analysed by RNA-Seq 454 sequence libraries and Affymetrix microarray.

Among the G protein gene family, *Gγ*'s are most diverse members and showed deletion in monocot as well as dicot Arabidopsis after speciation. The chromosomal rearrangements such as translocation was found for *GA1* and *Gγ-3* and most interestingly B copy of *GA1* showed 17nt insertion resulting in truncation of *GA1-B*. Most of the G protein gene family members showed upregulation or downregulation in response to biotic and abiotic stress conditions indicating that these members may have possible role in these type of signalling pathways which needs further investigation.

2.2. Introduction

Genes encoding heterotrimeric G protein complex subunits have been identified in a wide range of organisms including slime moulds, fungi, animals and plants (Jones and Assman, 2004). In animal models, seven transmembrane (7TM) G protein coupled receptors (GPCR) are bound to

the G protein heterotrimer complex consisting of Gα, Gβ, and Gγ subunits, and GDP is bound to Gα in its inactive state. The binding of the 7TM GPCR to the cognate ligand leads to the conformational change in GPCR, which results into the dissociation of the heterotrimeric complex from the receptor and to further dissociation of the Gα subunit from the Gβ-Gγ dimer due to the exchange of GTP with Gα bound GDP, through association with Guanine Exchange Factor (GEF). The GTP bound Gα and Gβ-Gγ dimer can readily interact with their target effector proteins. The GTPase activity of Gα can convert the bound GTP to GDP which enables the reconstitution of the heterotrimeric complex with bound GDP (Urano and Jones, 2014). However, the study in the Arabidopsis suggested that the G protein signalling pathways in plants do not require GPCR and can be activated by themselves; the Gα subunit in Arabidopsis, GPA1 maintains this self-activating mechanism by accelerating the guanine nucleotide exchange rate, without association with a GEF, and reducing the hydrolysis of GTP, relative to Gα found in animal cells, in absence of GPCR (Jones et al., 2011).

The G protein gene families had been identified in different plant species though there is diversity among them in terms of number of genes encoding the G protein subunits. Among plant species, Gγ's had been found to be more diverse subunits compared to Gα and Gβ. For example, the Arabidopsis genome encodes a single Gα (GPA1) and a single Gβ (AGB1), but encodes three Gγ subunits (AGG1, AGG2 and AGG3), whereas the rice genome has same number of Gα and Gβ, but encodes five Gγ subunits (Jones and Assman 2004; Li et al., 2012; Trusov et al., 2012). In soybean, there are two Gα, two Gβ and five Gγ encoding genes per diploid genome (Choudhury et al., 2011), while in *Brassica rapa* single Gα, three Gβ and five Gγ's had been identified (Arya et al., 2014) whereas there are five genes encoding Gγ's in tomato, with single Gα and Gβ encoding genes (Subramaniam et al., 2016). Gγ's are classified in three types, type A, B and C, based on the structural differences. The type A group include Gγ's that are small relative to other types, approximately to 100 amino acid (aa), and have a -CaaX motif at their C terminal end, where C is cysteine residue, aa are two aliphatic amino acid residues and X is any aa residue. Arabidopsis Gγ's, AGG1 and AGG2 belong to type A. The type B Gγ's do not have -CaaX motif, instead, most of the eudicot and monocot species have -SRxxKRWI and –KGSDFS as conserved motifs respectively. The type C Gγ's shares maximum similarity in the N-terminal and central regions with type A and B and they are comprised of varied length of cysteine rich C-terminal regions, which accounts for 19-38% of cysteine (Trusov et al., 2012). Besides these

three, the large unusual proteins having sequence similarity with Gα subunit have been found in the Arabidopsis genome and are called as extra- large GTP- binding proteins namely XLG1, XLG2 and XLG3. XLGs N=terminals have a stretch of nearly 400 aa with nuclear localization signal, while the C-terminal regions are similar to that of canolical Gα subunit. All these XLG's show the properties of Gα such as GTP binding and GTPase activity, however these activities are slower compared to Gα and require $Ca2^{+}$ as a cofactor (Ding et al., 2008). This suggests that beside heterotrimeric G proteins another class of GTP binding proteins is also present in the plant genome.

G proteins have been known to interact physically with different proteins that regulate the plant growth under normal, and abiotic stress conditions as well as in response to hormonal stress treatments; nevertheless the diverse network of G protein interactors give an indication that it act in coordination with the other hormone signalling pathways. The physical interaction of GPA1 with G protein coupled receptor (GCR), plastid protein thylakoid formation1 (THF1) and phospholipase Dα1 (PLDα1) have been reported in Arabidopsis (Pandey et al., 2004; Zhao and Wang, 2004; Huang et al., 2006). In bread wheat, *Triticum aestivum* Gα subunit (GA3) had been known to interact physically *in vivo* and *in vitro* with Clo3 and phosphoinositide-specific phospholipase C, PI-PLC1. Moreover an ortholog of *Ta*-Clo3 in Arabidopsis, Responsive to Dehydration (RD20) is known to be induced by ABA, drought and salinity stress (Khalil et al., 2011; Aubert et al., 2010). The interaction for the G proteins alpha subunit RGA1 with the protein chilling tolerance divergence 1 (COLD1) had been detected in rice; COLD1 is responsible for cold tolerance in japonica rice through calcium signaling (Ma et al., 2015). The rice COLD homolog in wheat cultivar Kenong199 have shown to interact with one of wheat G α subunit homeolog, *Ta*Ga-7A and regulate the plant height (Dong et al., 2019). The physical interactions of AGB1 with mitogen activated protein kinase 6 (MPK6) had been reported *in vivo* and *in vitro* (Xu et al., 2015) and this AGB1 has been shown to interact with brassinosteroid transcription factor BES1, and acts as positive regulator of the hypocotyl elongation in response to brassinolide treatment (Zhang et al., 2018). This suggests that G proteins are the essential component and their subunits act in regulation of hormonal signalling through interaction with other proteins.

The diverse role of G proteins in plant growth and development, defense against pathogens and hormonal signalling had been studied by mutant analysis. The mutant analysis in Arabidopsis showed that heterotrimeric G proteins regulate plant physiological processes including seed germination, silique and flower development, stomatal closure and movement, leaf morphology, seedling and root development and sugar sensing (Urano et al., 2013). Heterotrimeric G proteins have also been shown to regulate the seed and grain size, in studies with Arabidopsis and rice (Huang et al., 2009; Li et al., 2012). The Gβγ subunit have been shown to affect the fungal defense response against necrotropic plant pathogens like *Alternaria brassicicola* and *Fusarium oxysporum* via jamonate signalling in Arabidopsis, and Gβγ mutants were found to be susceptible to these pathogens (Trusov et al., 2006). G protein gene families have been shown to play regulatory roles in the hormonal signalling, by studies with ABA signalling (Pandey et al., 2006; Zhang et al., 2011) auxin (Ullah et al., 2003, Subramaniam et al., 2016), jasmonate (Trusov et al., 2006), and brassinosteroid signalling (Tsugama et al., 2013; Zhang et al., 2018). The rice Gγ's, RGG1 and RGG2, have also been known to be induced by cold, salt and drought stress (Yadav et al., 2012). These studies indicate that members of the heterotrimeric G protein gene families play direct or indirect role in the plant's response to biotic and abiotic stress conditions in different plants species.

T. aestivum, bread wheat, is among one of the most important cereal crops grown in the world in terms of human consumptions. It is a member of the tribe Triticeae and include approximately 300 species, which include the closely related species of bread wheat like *T. turgidum* (pasta wheat), *Hordeum vulgare* (barley) and *Secale cereale* (rye). *T. aestivum* is a hexaploid species and allopolyploidation through hybridization with species from *Aegilops* genus was major events in the evolution of Triticum species (Tsunewaki, 2009; Matsuoka, 2011). The divergence between the diploid species of *T. monococcum* and *T. urartu*, the progenitor and of the A genome occurred less than a million year ago (Huang et al., 2002). After the divergence of *T. urartu* and *T. monococcum*, hybridization between *T. urartu* and a species closely related to *Aegilops speltoides* (Taush) (SS genome) gave rise to the tetraploid species *T. turgidum* (AABB genome) and *T. timopheevii* (AAGG genome) by two independent hybridization events in the period less than 0.5 million year ago. Nearly 10,000 years ago, the second hybridization event between *T. turgidum* (AABB genome) and wild wheat species *Aegilops tauschii* (DD genome) gave rise to the current bread wheat *T. aestivum,* which has AABBDD genome (Matsuoka, 2011).

The hexaploid bread wheat is one of the important staple foods grown all over the world, being consumed by 30% of world population, has massive genome of size 17 gigabase (Eversole et al., 2014). The climate change has been a global concern in recent years and the increase in the temperatures are correlated with the incidences of biotic and abiotic stresses, which severely decrease crop yield and growth of the plants. The combined occurrence of the abiotic stresses like drought and heat at different growth stages of plants are found to be more devastating than a single stress (Pandey et al., 2017). In order meet the demand for food in the near future, it will be important to understand the underlying mechanism of plant tolerance of stress conditions. Characterization of gene families and the expression analysis studies using RNA-Seq in wheat will be a prerequisite for studying the response of genes to different stress conditions.

I have identified the members of the G protein gene families in *Triticum aestivum*, analysed their tissue specific expression pattern during the course of development and expression under abiotic and biotic stress conditions using the available transcriptome and microarray data. The identification of conserved domains among different gene family members and their evolutionary relationship with other monocot species and dicot Arabidopsis was carried out. This study will give an insight into the response of G protein gene families to abiotic and biotic stresses that can be further used to study the functional genomics of G proteins in *T. aestivum.*

2.3. Materials and Methods:

2.3.1. Compilation of full length gene sequences for wheat heterotrimeric G protein gene family members

The coding sequences for Gα (Ta-*GA1*) and Ta-*Gβ* subunits (Accessions: HQ020506.1 and AB090160.1) in *T. aestivum* and the sequences of the five heterotrimeric Gγ subunits, *Os*GGA (γ1), *Os*GGB (γ2), *Os*GGC1 (γ3), *Os*GGC2 (γ4) and *Os*GGC3 (γ5) from rice (Trusov et al., 2012) were retrieved from NR database of NCBI. The sequences were used to search in TSA and EST database at NCBI to obtain homologous sequences in the Triticum species, *T. aestivum*, *T. urartu* and *T. turgidum*. Multiple independent EST sequences that shared at least 99% identity were used to form contig using CAP3 (doua.prabi.fr/software/cap3), in order to generate the full length sequences for the coding regions of respective heterotrimeric G protein encoding genes. The cDNA sequences for the heterotrimeric G proteins were used for BLASTn search in the

International Wheat Genome Sequence Consortium (IWGSC) genomic survey sequence database RefSeq v1.0 (http://www.wheatgenome.org/), and the genomic sequences for high scoring hits from the A, B and D homeologous chromosomes were selected. The respective genomic sequences obtained for homeologous gene copies at IWGSC were used iteratively to retrieve full length cDNA sequences for the corresponding cDNA sequences in the TSA and EST databases, which were not identified in the initial screening of those databases. The translation for each FL cDNA sequence to its corresponding amino acid sequence was carried out using ExPASy translate tool (web.expasy.org/translate/).

2.3.2. Identification of gene sequences for heterotrimeric G proteins in other monocot species

The G protein sequences from *T. aestivum* were used to search for sequences in monocot species of Triticeae tribes which include *Aegilops tauschii*, *Hordeum vulgare* and *Secale cereale*, and other species *Brachypodium distachyon* and *Setaria italica*, in the NR, EST and TSA databases at NCBI by tblastn. The G protein sequences from rice were used to search for homologs in monocot species *Zea mays* and *Sorghum bicolor*. If the sequences were not found in EST or TSA then PlantGDB database for respective plant species was used to identify genes that appeared to be missing from gene families among closely related sequences (http://www.plantgdb.org/). The sequences for G proteins genes in Arabidopsis were retrieved from TAIR (https://www.arabidopsis.org/).

2.3.3. Conserved domain and phylogenetic analysis

The conserved domains for G protein gene families from *T. aestivum*, other nine monocot species and Arabidopsis were confirmed by Batch Conserved Domain Search tool (Batch CD search tool)(https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) at NCBI and domain architecture was analysed using Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/). The multiple sequence alignment for proteins was done by Clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and the conserved domains and motifs for different subunits were identified.

Phylogenetic analysis of heterotrimeric G proteins gene families for GA1, Gβ and Gγ's subunits for ten plant species (*T. aestivum, H. vulgare, Ae. tauschii, Z. mays, A. thaliana, B. distachyon, Sorghum bicolor, Setaria italica, Secale. cereale* and *O. sativa*) was carried out using full-length amino acid sequences by MEGA7 as described by Khalil et al., 2011. This analysis will give an idea about the relationship about the divergence of the GA1 and Gβ in monocot species and Arabidopsis over the period of time. In case of the Gγ's, it will determine the recent addition or deletion in the paralogs among the species. In total, ten sequences for both GA1 and Gβ were used in the analysis. Phylogenetic analysis for Gγ1 and Gγ2, and Gγ3, Gγ4 and Gγ5 were separately carried out where 20 and 21 sequences were used respectively. The three homeologs in Triticum are nearly identical hence the A homeolog of each G protein gene from *T. aestivum* was used as a representative in phylogenetic tree construction. The amino acid sequences for each subunit were aligned using MUSCLE in MEGA7 (Kumar et al., 2016) and the phylogenetic trees were constructed using Whelan and Goldman (WAG) model by Maximum likelihood method, with discrete gamma distribution 5 categories (+G, parameter =1.5141, 0.6463 ,2.6948 and 1.5231) for GA1, Gβ, Gγ1 and Gγ2, and Gγ3, Gγ4 and Gγ5. respectively. All positions with less than 95% site coverage were eliminated and alignment gaps less than 5% of the total length, missing data, and ambiguous bases were allowed at any position and 100 bootstraps were used for tree constructions.

2.3.4. Exon/Intron structure determination

To determine the exon/intron regions, the coding sequences for the *T. aestivum* Ta-*GA1*, Ta-*Gβ* and Ta-*Gγ* subunits were compared to the genomic sequences obtained from IWGSC database. The exon and intron lengths were determined by using Splign (https://www.ncbi.nlm.nih.gov/sutils/splign/ splign.cgi? textpage=online&level=form) and were confirmed manually for the presence of intron beginning and ending consensus sequences GT …. AG. For other monocot species and Arabidopsis, the coding sequences for G protein subunits were compared with their respective whole genome shotgun sequences available at NCBI database. The diagram for exon/intron structure in *T. aestivum* was generated using Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/).

2.3.5. Bimolecular fluorescence complementation, intracellular and localization of *T. aestivum* Gβ and Gγ's

Protein-protein interaction was assayed by bimolecular fluorescence complementation. pDONR207 entry clone for *Gβ*-A was recombined with Gateway YFP plant expression vector, sYFP-N (pCL112_JO) (received from Dr. Alan Jones), and pDONOR207 entry clone containing *Gγ1*-A and *Gγ2*-A were recombined with sYFP-C (pCL113_JO) to test for protein-protein interaction. For intracellular localization of the G proteins independently of the interaction assay by BiFC, entry clones for *Gβ*, *Gγ1* and *Gγ2* in pDONR201 were recombined with Gateway GFP plant expression vector PK7FWG2 (Karimi et al., 2002) using Gateway LR reactions. The destination vectors were transformed into electrocompetent *Agrobacterium tumefaciens* strain AGL1. The p19 plasmid was used to supress the gene silencing during gene expression in plants. The individual cultures for *Gβ*, *Gγ1* and *Gγ2*, p19 and a plasma membrane (PM) marker-RFP fusion were grown overnight. For BiFC, one each volume of cultures, at OD600 of 0.5, carrying *Gβ*, *Gγ1* and *Gγ2* and p19 were mixed , which was further mixed with one volume of overnight cultures of plasma membrane marker diluted to an OD600 of 0.005, 0.01 and 0.02 in separate samples. For intracellular localization with fusions to FL GFP, the individual test cultures and p19 at OD600 of 0.5 and the PM marker at the same OD were mixed in separate tubes. The 15 ml tubes were centrifuged at 4000g for 20 min, and the pellet obtained was suspended in 3 ml Agroinfiltration solution containing 10 mM $MgCl_2$, 150 µM acetosyringone and sterilized distilled water. The mixed cultures were kept at room temperature for 4hrs and then suspensions were used to infiltrate the abaxial leaf surface of 4-5 week old *N. benthamiana* plants. Plants were kept for 28 hrs. at 21-24^{o}C under long day conditions in a greenhouse followed by imaging using confocal microscope in Centre for Microscopy and Cellular Imaging at Concordia University.

2.3.6. Gene expression analysis in tissues and in response to stress

RNA-Seq raw read datasets for five different tissue types and stages were analyzed. These included the seed at whole plant fruit ripening stage, root at cotyledon emergence, leaf at whole plant seed formation stage 30-50% moisture, stem and inflorescence tissues. The datasets for five different tissue types and stress conditions osmotic, heat, and combined osmotic and heat stress was obtained from RNA Seq datasets repository available in the SRA database at NCBI, whereas for cold stress and biotic stress *Fusarium graminearum infection*, the datasets were obtained from the Array Express (https://www.ebi.ac.uk/arrayexpress). Gene expression across a panel of seventy

one different tissue types in Azhurnya spring wheat was analysed by wheat eFP browser (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi). The identifiers for the datasets the raw reads used in the gene expression analysis of G protein gene family members are given in **Supplementary Table S2.1.**

The FASTQ files for the raw datasets were collected using European Nucleotide Archive (ENA) search (https://www.ebi.ac.uk/ena) and were converted to FASTA format using FASTX Toolkit 0.0.13.2 (http://hannonlab.cshl.edu/fastx_toolkit/links.html). The RNA-Seq transcript alignment for 3' UTR regions of the Ta- GA1 and Ta-Gβ and Ta-Gγ2 cDNA sequences were used, because the coding regions were too similar to readily distinguish between homeologous sequences. The nucleotide sequences comprising the coding regions and the 3' UTR for Ta-*Gγ1*, *-3*, *-4*, and *-5* were used with CD-HIT-EST-2D algorithm (Fu et al., 2012) to measure levels of expression. The parameters used were, word size of 5 (n=5) and similarity cut-off of 99% (-c=99), which could easily distinguish between the A, B and D homeologs. The details describing the regions and nucleotide length of the sequences used for the alignment in RNA-Seq analysis are given in the **Supplementary Table S2.2.**

The relative level of expression in eleven different tissues (germinating seed coleoptile, germinating seed root, germinating seed embryo, seedling root, seedling crown, seedling leaf, immature inflorescence, floral bracts before anthesis, pistil before anthesis, anthers before anthesis, 3-5 DAP caryopsis, 22 DAP embryo, 22 DAP endosperm) were determined by 61K Affymetrix microarray datasets from Schreiber et al. 2009, available at PLEXdb database (http://www.plexdb.org/). Similarly, the change in gene expressions in response to drought, heat and combined stresses in two *T. durum* wheat cultivars, Ofanto and Cappeli (Aprile et al., 2013), and in response to cold treatment in wheat cultivars of winter habits (winter Norstar and winter Manitou) and spring habits (spring Norstar and spring Manitou) were determined from the Affymetrix microarray data available at PLEXdb database (Laudencia-Chingcuanco et al., 2011). The gene identifiers for the Affymetrix microarray are given in **Supplementary Table S2.3.**

Two-way ANOVA was used for analysis of factorial experiments, to compare different genotypes under different treatment conditions. The significant genotype by treatment interaction effect in two-way ANOVA indicates that genotypes responded differently to the treatment. The statistical significance for differences in the relative level of expression of genes in different

tissues and the gene expression in response to stress were analyzed by one way ANOVA followed by Duncan's multiple range test.

2.4. Results

2.4.1. Heterotrimeric G Protein genes in *T. aestivum*

The great majority of the sequences identified for genes encoding heterotrimeric G proteins were confirmed with three independent databases, which included transcriptome shotgun assembly (TSA), expressed sequence tag (EST) database at NCBI and International Wheat Genome Sequencing Consortium (IWGSC) database of genomic chromosomal survey sequences (IWGSC). In cases of disagreements between the databases, sequences that could be confirmed by at least two independent data sources were selected. The majority of genes of the heterotrimeric G protein families were represented by three to six sequences in the EST database. The wheat chromosomal survey genomic sequence database (IWGSC whole genome assembly RefSeq v1.0) at IWGSC had sequences, which covered the full length coding regions for three *GA1*'s and three *Gβ*'s and 12 *Gγ* genes in *T. aestivum* and facilitated the chromosomal assignment of the gene family members and confirmed the accuracy of the sequences.
The heterotrimeric G proteins gene families in *T. aestivum* consist of single *GA1* and *Gβ*, and four *Gγ* genes per haploid genome, and with three homeologs for each gene in the hexaploid genome. Thus, three *GA1*, three *Gβ* and 12 *Gγ* full length cDNA sequences were identified in *T. aestivum*. The protein lengths for Ta-GA1 varied between 367-385 aa, Ta-Gβ's were 380 aa and the most diverse family were Ta-Gγ's which ranged from 97 to 305 aa. Gγ1 and -4 are the shortest and longest Ta-Gγ's with 97-98 aa and 285-305 aa respectively. The sequences and the identifiers for G protein gene families in *T. aestivum* are given in **Supplementary Table S2.4** and **S2.5.**

The NR database of NCBI genbank had representation for three *T. aestivum* Gα sequences, which include TaGA1 (AB090158.2), TaGA2 (AB090159.1) and TaGA3 (HQ020506.1), but the comparison of these sequences by alignment showed that these sequences have large regions of 100% sequence identity but with some gaps in the alignment. Only TaGA3 sequence was in 99-100% agreement with the TSA and EST database, showing the accuracy of this sequence and suggesting that TaGA1 and TaGA2 sequences may have some sequencing errors. We named the

T. aestivum Gα's identified in this study as Ta-*GA1* and other species Gα's as *GA1*. The blastn search for Ta*GA1* at IWGSC database showed that the homeologous copies for these genes were located on the chromosome 1BL, 7DS, and 7AS. The IWGSC derived sequences for Ta-*GA1* homeologs agreed with the TSA and ESTs, whereas the sequences from 7BS and unknown scaffold had no representation with 99-100% identity in the TSA and EST database, and these hits did not contain full CDS for these genes. Surprisingly, the B homolog of Ta*GA1* was found to be on the long arm of chromosome 1, instead of chromosome 7B, nevertheless, A and D copies for Ta-*GA1* were found to be located on the short arms of chromosomes 7A and 7D respectively. Support for the single Ta-GA1 subunit can be derived from the comparison with other monocot species like *Ae. tauschii*, rice, sorghum, barley, *B. distachyon*, maize, rye and foxtail millet; all of these species have single gene encoding the GA1 subunit.

The three homeologous copies for Ta-*Gβ* derived from TSA and EST database agreed with the genomic sequences in the IWGSC whole genome sequence database. There are four genes encoding Gγ subunits per diploid wheat genome; each gene family member has three homeologous copies. Most of the Ta-*Gγ* gene coding sequences derived from the TSA and EST databases were agreed with the genomic sequences in the IWGSC database, though Ta-*Gγ1*-B and three homeologous copies of Ta-*Gγ3* had no representation in the EST database or TSA databases. Most of the *Gγ* homeologous gene copies were located on the corresponding short or long arm of A, B and D homeologous chromosomes except for Ta-*Gγ3*. The A and D homeologs of Ta-*Gγ3* were located on the short arm of chromosomes 7AS and 7DS, respectively, whereas the third homeolog was located on chromosome 4AL. This is likely due to the reciprocal translocation between chromosomes 4A and 7B, which had been previously reported in the wheat genome (Devos et al., 1995).

2.4.2. Heterotrimeric G Proteins in monocot species

Single GA1 and Gβ subunit for G protein gene families were found in all the nine monocot species used in this study and most of the sequences were confirmed with at least two independent databases. The *GA1* and *Gβ* cDNA sequences identified for *Ae. tauschii*, *H. vulgare*, *B. distachyon*, *S. italica, S. bicolor* and *Z. mays* in NR database agreed with TSA database and most of them were agreed with PlantGDB database for respective species, and with full-length or partial length support in the EST databases for respective species. The exception was rye, whose

sequences were solely based on the TSA database at NCBI. In monocot species, a minimum of three and up to a maximum of five Gγ's were found per diploid genome. Four full length cDNA sequences for *Gγ*'s were identified in each *B. distachyon* and *H. vulgare*, and three *Gγ*'s were identified in *Secale cereale*, while monocot species *S. bicolor* and *Z. mays* each had five *Gγ*'s. The details for the identifiers and sequences for the respective monocot species in different databases are given in **Supplementary Table S2.6 and S2.7**.

2.4.3. Conserved domains in G protein gene families

The conserved domains and domain architecture for the G protein subunits were determined by Batch CD search tool and SMART. These results were confirmed by multiple sequence alignment of G protein subunits and identification of functional domains and motifs. Multiple sequence alignment of the GA1 subunit from *T. aestivum* and other species showed that these subunits had conserved glycines at a conserved myristolation consensus sequence and cysteine S-acetylation sites. These modifications function in the localization of GA1 to the plasma membrane; it has been reported that mutation in one of these residues could mistarget the protein to the cytosol (Adjobo-Hermans et al., 2006). Batch CD search tool search with GA1 subunit from *T. aestivum*, monocot species and Arabidopsis showed that these subunits have conserved functional G1 to G5 motifs, of which G1 (GAGESGKS) is P-loop that has function in NTP binding, G2 has conserved threonine residue (T) which is responsible for conformational change of GA1 protein, G3 (DxxG) is involved in GTP hydrolysis and G4 (NKxD) is involved in guanine recognition (Temple and Jones, 2007). The details for the conserved domains and motifs for GA1 in *T. aestivum* and other species are given in **Supplementary Figure S2.1.**

Gβ subunits have approximately 40aa conserved motifs that ends with Tryptophan-Aspartic acid (WD) and are known to be responsible for 7 bladed β propeller structures of Gβ. Seven conserved motifs that ends with WD were detected in Gβ's of *Triticum* and other monocot species. The details for the conserved motifs for Gβ's are given in **Supplementary Figure S2.2.**

Gγ's had been categorised as type A, B and C based on the sequence lengths and the conserved domains (Trusov et al., 2012). Type A Gγ's are smaller than the other classes and have post translation prenylation at the C-terminal CaaX motif, where C is cysteine residue, aa are two aliphatic amino acids and X may be methionine, glutamine, alanine, cysteine, or serine residues

(Trusov et al., 2012). Multiple sequence alignments showed that Gγ1 in *T. aestivum* and other monocot species had –CWFL, whereas Arabidopsis Gγ1 and Gγ2 had –CLIL and -CSIL as a CAAX motif respectively. Gγ2's in monocots have been reported to have a C-terminal conserved –KGSDFS (Trusov et al., 2012), however *T .aestivum*, *H. vulgare* and *S. cereale* had –KGSDFA. We categorised *T. aestivum* Gγ-1 and -2 as type B, which are also known as non prenylated Gγ subunits. The third type Gγ's, Type C, are longer than other two classes and have a cysteine rich region in their long C terminal ends. *T. aestivum* Gγ-3, -4 and -5's fall into this category. Gγ's have highly conserved –DPLL motif, which functions in the formation of hydrophobic contact with Gβ (Temple and Jones, 2007). The -DPLL motif was found to be conserved in the Gγ-1 and Gγ-2's, whereas in Gγ-3, -4 and -5, the first phenyl-alanine was replaced by leucine or methionine, and second phenyl-alanine was replaced by isoleucine in *T. aestivum*, monocots and Arabidopsis. The details for the conserved motifs for Gγ's are given in **Supplementary Figure S2.3 and S2.4.**

2.4.4. Exon/Intron structure in *T. aestivum* and other species

Genes encoding GA1 subunits in *Ae. tauschii* and A and D homeologous copies of *T. aestivum* had 12 exons each, however, the Ta-*GA1*-B homeolog has 13 exons, due to an additional intron inserted into wheat corresponds to exon 11 in the Ta-*GA1* homeologs. Six exons were found in *Gβ*'s in *T. aestivum*, other monocots and Arabidopsis. *Gγ* genes have a similar number of exons numbers in *T. aestivum*, other monocot and Arabidopsis, of which, *Gγ-1* and *-2* had four exons each, whereas *Gγ-3, -4* and *-5* had five exons each, moreover, the length of the third exon in Triticum Gγ's was found to be conserved. The details for exon-intron structure in *T. aestivum* are given in the **Figure 2.1 and Supplementary Table S2.8** and number of exons for other monocot species is given in **Supplementary Table S2.9.**

2.4.5. BiFC and intracellular localization of *T. aestivum* Gβ and Gγ's

Ta-Gβ was shown to interact *in vivo* in the leaves of *N. benthamiana* with Ta-Gγ1 and Ta-Gγ2, 28-32 hrs after infiltrating the leaves with Agrobacterium carrying the BiFC constructs. The interaction was localized to the plasma membrane. The in vivo positive interactions for these proteins showed that the genes identified indeed codes for the heterotrimeric G protein subunits in Triticum and are localized to the previously reported localization of such proteins. The

localization of Ta-Gβ, Ta-Gγ1 and Ta-Gγ2 subunits were assayed independently of the BiFC assay by expressing the proteins transiently as fusions to full length GFP in the leaf pavement cells of *N. benthamiana*. Each of this subunit was localized to plasma membrane. The details are for the interaction and intracellular localization are shown in **Figure 2.2.**

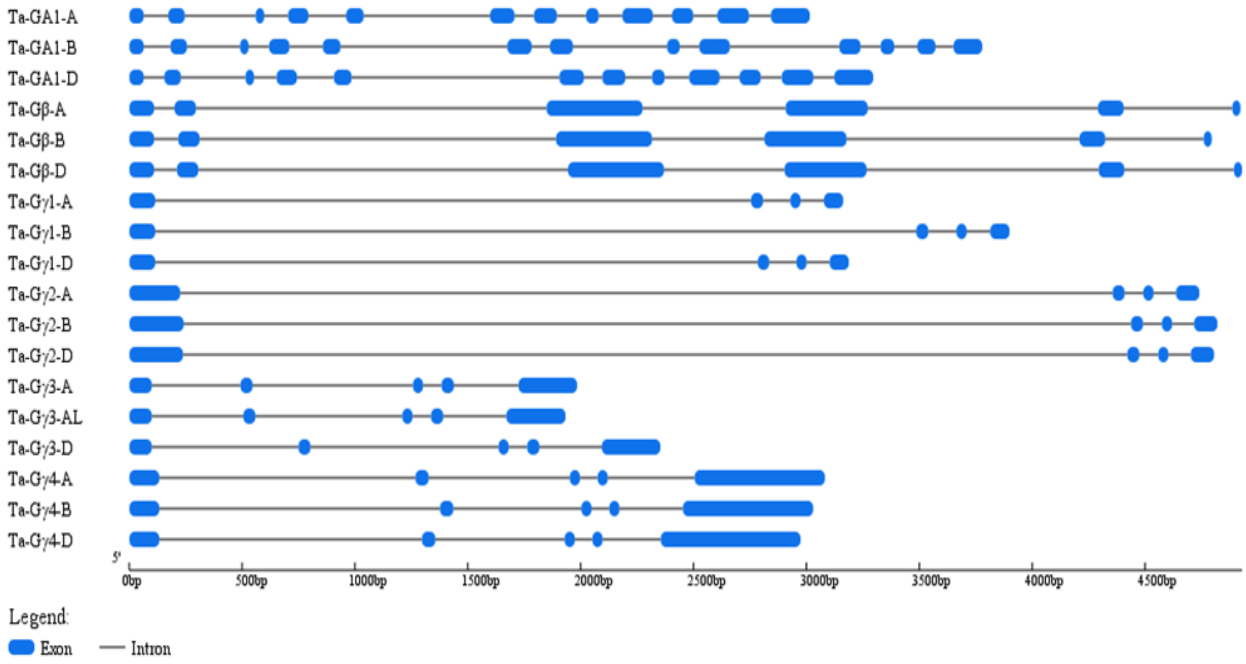

Figure 2.1. Exon/Intron structure for G protein gene family members in *Triticum aestivum*. Blue rectangles and lines indicate exons and introns respectively.

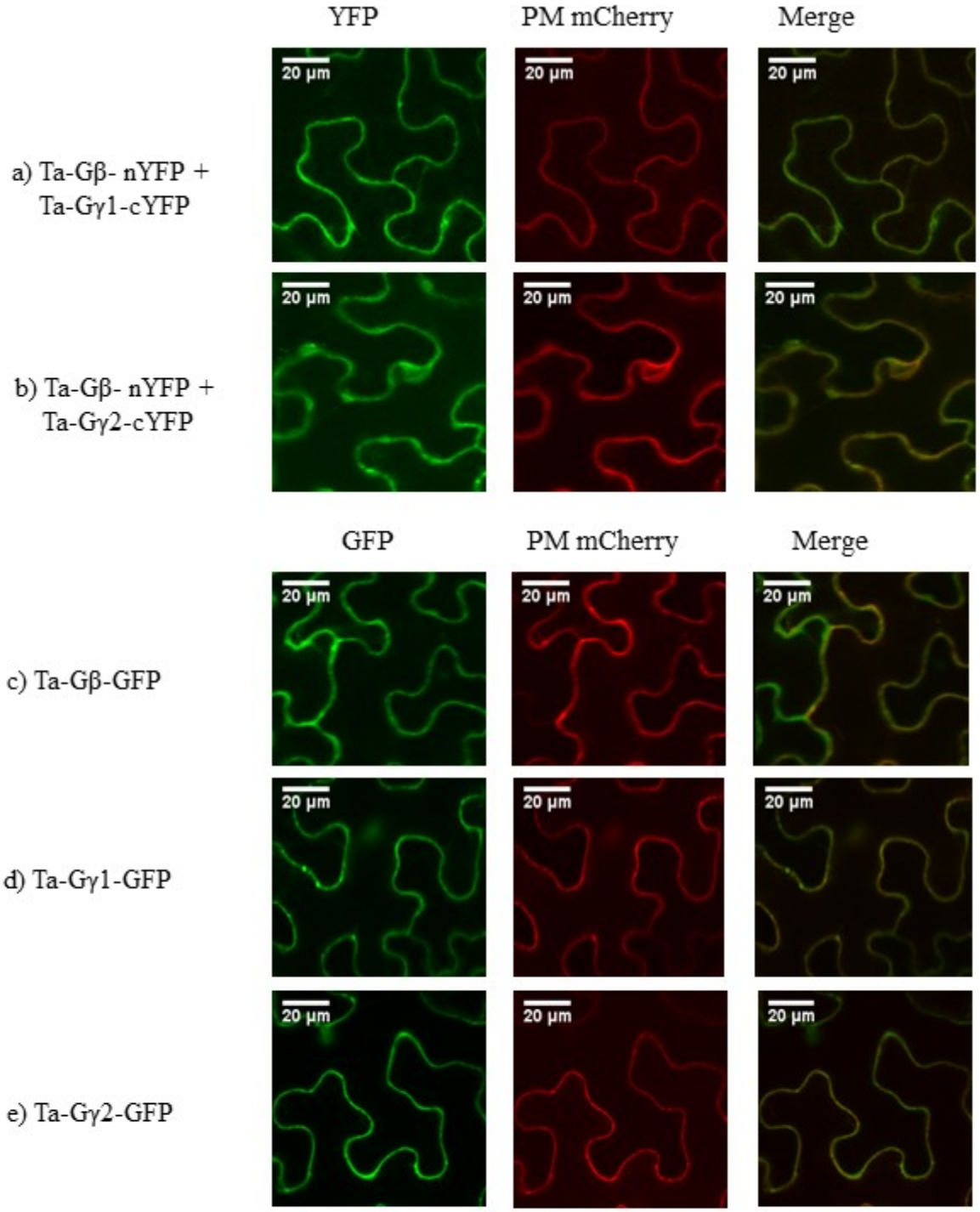

Figure 2.2. BiFC and intracellular localization of Gβ, Gγ1 and Gγ2. In vivo protein-protein interaction of *T. aestivum* a) Gβ-Gγ1 and b) Gβ-Gγ2 by BiFC and intracellular localization of c) Gβ, d) Gγ1 and e) Gγ2 using full length tagged GFP; Scale bar = 20 µm. The first panel indicate the YFP or GFP, middle panel PM-mCherry indicate the plasma membrane marker which is the plasma membrane aquaporin PIP2A in Arabidopsis and the merge indicate that the two proteins were localized to the plasma membrane.

2.4.6. Tissue specificity of G protein gene family members in *T. aestivum*

The relative level of the gene expression in the different tissue types across the panel of seventy one tissues at different stages of plant growth and development in Azhurnya spring wheat was analysed by transcriptome dataset available at eFP Browser (bar.utoranto.ca (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi)/. G protein gene families showed the varied tissue specific expression patterns in the transcriptome and microarray analysis. The gene expression level analysed in Azhurnya spring wheat was ranged from the highest value of 42.13 RPKPM for *Gγ2*-B to the undetectable levels for *Gγ1*-B and *Gγ4* homeologs. Though the differences in the gene expression levels of A, B and D homeologous copies among gene family members were detected, in most of the cases the differences were less than 2 fold, except for *GA1* and *Gγ1*. The gene expression for *GA1*-D was higher than it's A and B genome homeologs and had more than 2 fold differences in nearly approximately 75% of the tissues. *Gγ1*-A and -D genome homeologs had similar levels of expression in most tissues, but the B genome homeolog had much lower levels of expression and was undetected in most of the tissues assayed. The level of expression for *GA1* and *Gβ*'s showed nearly a continuous variation in the level of expression among the 71 tissues; the level of expressions did not show more than two fold difference from level observed in the tissue with the median level of expression in over half of the tissues in the studies. *Gγ2* also showed relatively high levels of expression with little variation over a wide range of tissues. Other members of the *Gγ* gene family showed lower levels of expression compared to *Gγ2*, and a greater degree of variation between tissues.

The *GA1*'s and *Gβ*'s were most highly expressed in the shoot apical meristem at seedling stage, shoot axis at different stages, spike and fifth leaf blade. In addition, *Gβ* was highly expressed in stigma and ovary tissues with the value ranged from 12.74-14.48 RPKM (**Supplementary Table S2.10**). In Gγ's, the higher level of gene expression for *Gγ1*-A and –D in root tissues at different stages of development were detected, whereas in most of the tissues the expression for *Gγ1*-B was undetectable. The flag leaf sheath and blade tissues showed relatively high level of expression for *Gγ2* and *Gγ4* homeologs; whereas *Gγ1*, *-3* and *-4* were undetectable in some of the tissues. *Gγ3* was the paralogous gene set with the lowest levels of expression among gene family members; with the expression levels ranged between 3.84 RPKM to undetectable. Tissues with the highest relative levels of expression included the shoot apical meristem and shoot axis at

different developmental stages. The details for the gene expression in the different tissue types across the panel of seventy one tissues in Azhurnya spring wheat is given in **Supplementary Table S2.10**.

Two other transcriptome studies that were carried out with a smaller number of tissue types confirm the trends observed in the Azhurnya spring wheat. Datasets of Pingault et al. 2005 characterized the transcriptome in five tissues types, namely the seed at whole plant fruit ripening stage, root at cotyledon emergence, leaf at whole plant seed formation stage 30-50% moisture, stem and inflorescence. Specific analysis of these datasets for the gene expression level for G protein gene families showed a range of expression from 9.50 RPKPM to undetectable. The gene expression analysed was largely in consistent with the results from seventy one tissues in Azhurnya spring wheat. This analysis also showed higher expression for *GA1*-D compared to its homeologs in all five tissue types. The high level of expression in all the five tissue types was detected for *Gβ*'s. *Gγ2*'s were highly expressed paralogous groups among the *Gγ*'s in all five tissue types. *Gγ1* was relatively highly expressed in roots at cotyledon emergence, which was in agreement with expression analysed in Azhurnya spring wheat. *Gγ3* was the paralogous gene set with the lowest level of expression, also consistent with gene expression analysed in Azhurnya spring wheat. *Gγ4*'s had their highest level of expression in the stem and inflorescence tissues, an observation in addition to the higher gene expression in fifth leaf sheath tissue at five leaf stage seen in the Azhurnya spring wheat analysis noted above. The details for transcriptome analysis in the five different tissues types from datasets by Pingault et al. 2005 are given in **Figure 2.3 and Table 2.1.**

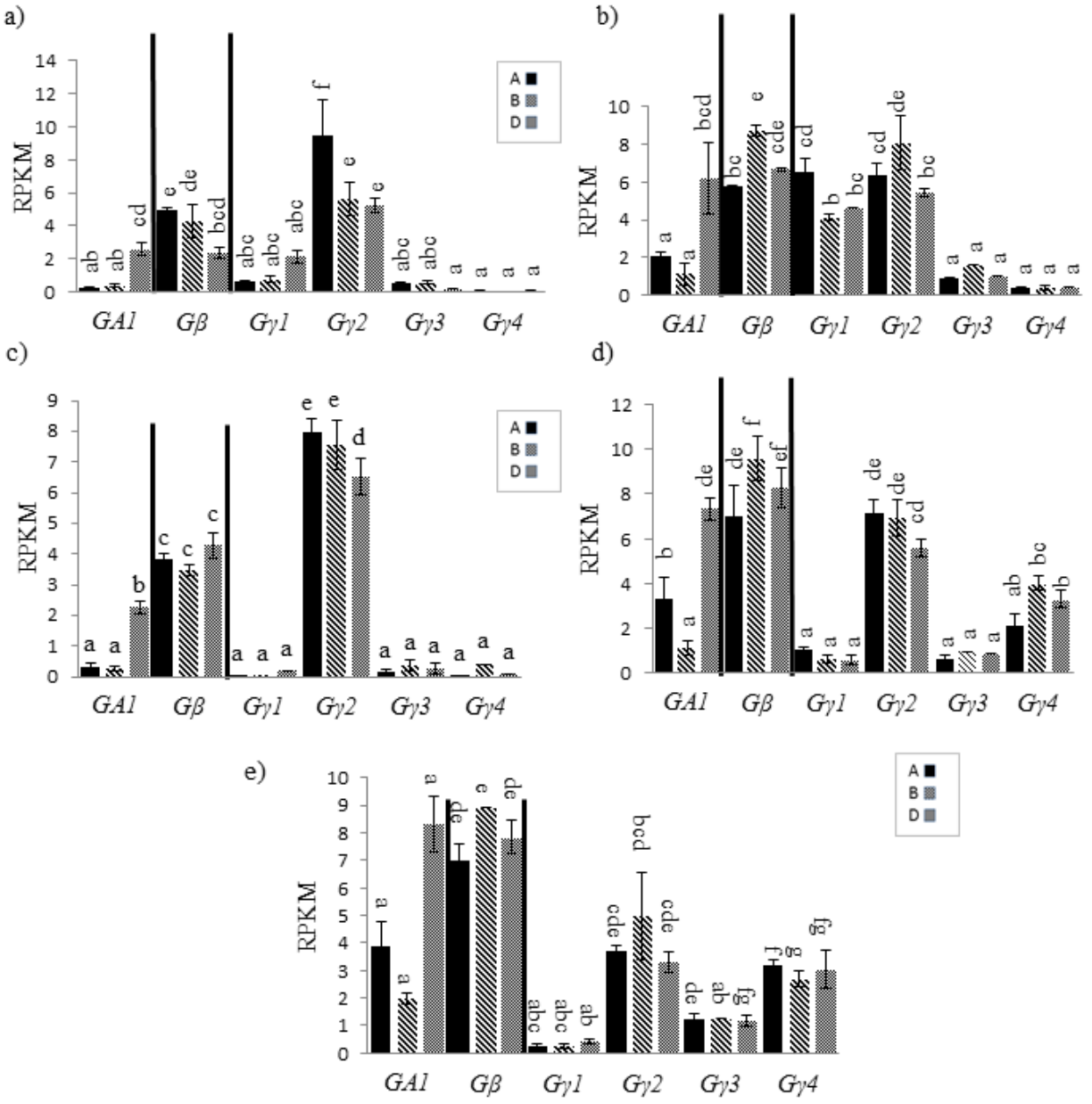

Figure 2.3. Tissue specific expression for G protein gene families analysed in five different tissue types by RNA-Seq. Gene expression of heterotrimeric G protein gene families measured in five different stages and tissues of *T. aestivum*, including a) whole plant ripening stage fruit b) cotyledon emergence root c) leaf d) stem and e) inflorescence. The expression values are represented as reads per kilo base per million (RPKM). RPKM values are based on the two replicates each. The significance of differences in the gene expression is estimated using one way ANOVA. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

Table 2.1. Tissue specific expression of G protein genes family members in five different *T. aestivum* tissues by assayed by RNA-Seq

Gene	Expression in RPKM					Fold change relative to leaf tissue				
	[a]Leaf	[b]Seed	[c]Stem	[d]Root	[e]Inflorescence	[a]Leaf	[b]Seed	[c]Stem	[d]Root	[e]Inflorescence
GA-1-A	0.34	0.29	1.04	2.09	0.25	1	0.84	3.02	6.11	0.73
GA-1-B	0.26	0.39	0.60	1.12	0.23	1	1.50	2.29	4.26	0.86
GA-1-D	2.26	2.58	0.55	6.21	0.42	1	1.14	0.24	2.75	0.18
Gβ-A	3.82	4.99	7.17	5.82	3.70	1	1.31	1.88	1.52	0.97
Gβ-B	3.47	4.29	6.92	8.73	4.96	1	1.24	2.00	2.52	1.43
Gβ-D	4.28	2.36	5.58	6.67	3.30	1	0.55	1.30	1.56	0.77
Gγ1-A	0.02	0.64	0.56	6.56	1.24	1	36.49	32.07	373.72	70.84
Gγ1-B	0.04	0.75	0.95	4.14	1.25	1	18.31	22.97	100.54	30.45
Gγ1-D	0.19	2.14	0.82	4.61	1.17	1	11.44	4.38	24.67	6.27
Gγ2-A	7.98	9.51	2.10	6.36	3.16	1	1.19	0.26	0.80	0.40
Gγ2-B	7.55	5.59	4.00	8.08	2.69	1	0.74	0.53	1.07	0.36
Gγ2-D	6.52	5.25	3.28	5.41	3.05	1	0.80	0.50	0.83	0.47
Gγ3-A	0.15	0.50	3.29	0.92	3.88	1	3.29	21.74	6.10	25.64
Gγ3-B	0.37	0.59	1.10	1.59	1.98	1	1.60	3.00	4.32	5.38
Gγ3-D	0.27	0.19	7.34	1.00	8.31	1	0.70	26.93	3.66	30.46
Gγ4-A	0.02	0.07	6.99	0.41	7.00	1	3.97	370.82	22.00	371.06
Gγ4-B	0.42	0.04	9.58	0.41	8.92	1	0.11	22.81	0.97	21.24
Gγ4-D	0.08	0.10	8.30	0.44	7.83	1	1.20	103.45	5.48	97.68

Note: Tissues at different stages include [a]Leaf : whole plant seed formation stage 30-50% moisture, [b]Seed: whole plant fruit ripening stage, [c]Stem : two nodes or internode visible stage, [d]Root: cotyledon emergence stage, [e]Inflorescence: maximum stem length stage. Values are expressed in Reads per kilo base per million (RPKM). Fold change is calculated relative to [a]Leaf considering expression equal to 1.

The tissue specific expression across a panel of thirteen different tissue types at different stages of development was also analysed on the 61k wheat Affymetrix microarray from the datasets by Schreiber et al. 2009. Though the microarray does not distinguish between the homeologous copies of the gene, it was in fair agreement with the transcriptome analysis by RNA-Seq for the tissue specific expression of G protein gene families. *GA1* and *Gβ* were highly expressed in the germinating seed, coleoptile and embryo, and immature inflorescence. The highest relative levels of expression for *Gγ1* was detected in root tissues at germination seed and seedling stage, whereas *Gγ2* was expressed in all the tissues but with the lowest levels of expression in anthers and pistils before anthesis compared to other tissues which agreed with the RNA-Seq data for Azhurnya discussed above. *Gγ3* showed the lowest level of expression among the *Gγ* paralogs on 61K Affymetrix microarray also; however it was highly expressed in anthers before anthesis, which is consistent with the RNA-Seq analysis. Interestingly, *Gγ4* was highly expressed in germinating seed coleoptile and in the inflorescence. The details of tissue specific expression using microarray data analysis are given in **Figure 2.4, Figure 2.5 and Supplementary Table S2.11.**

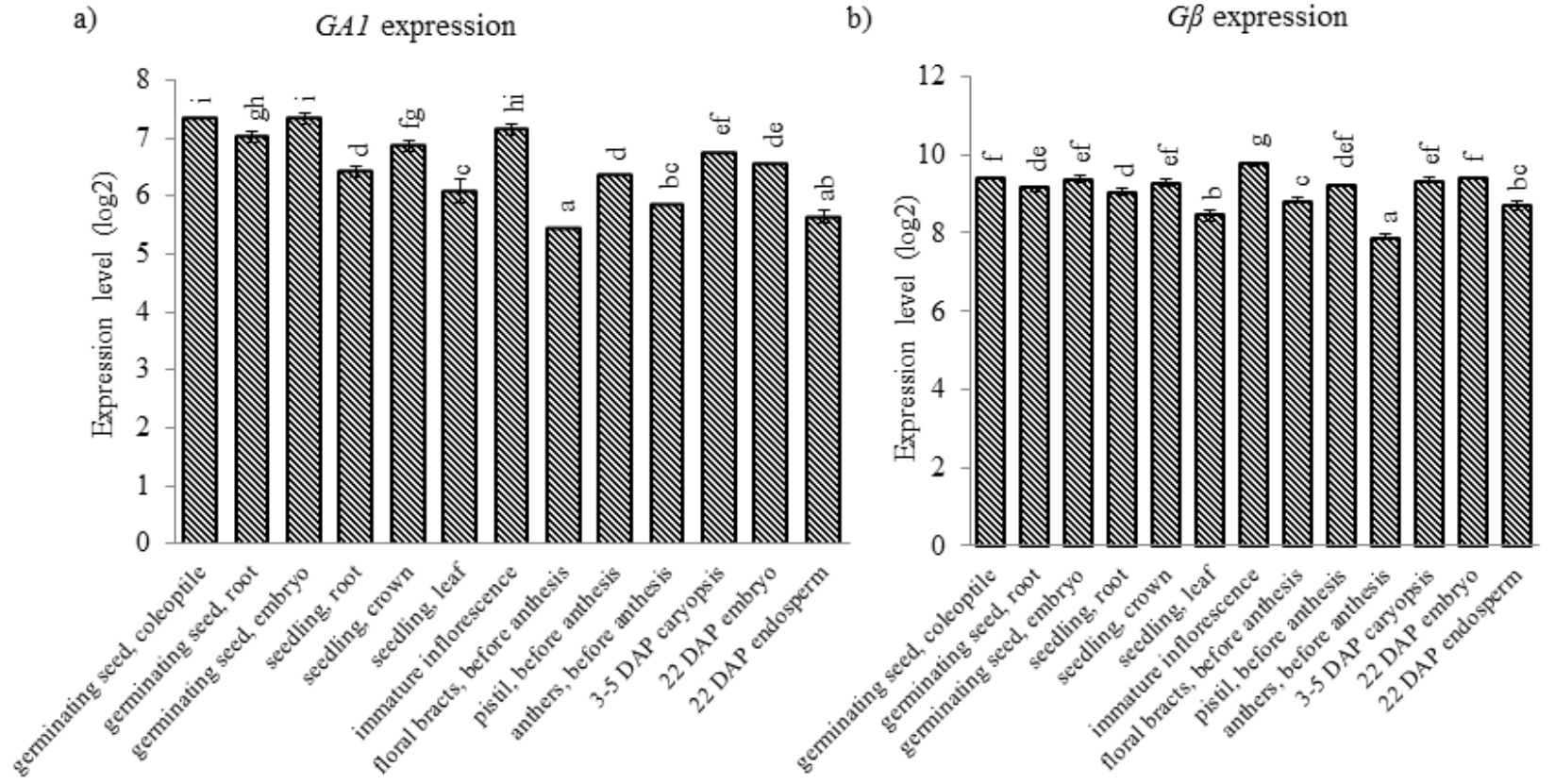

Figure 2.4. Tissue specific gene expression relative level of *GA1* and *Gβ* by microarray. a) *GA1* and b) *Gβ* in *T. aestivum* were measured across a panel of thirteen different developmental stages (germinating seed coleoptile, germinating seed root, germinating seed embryo, seedling root, seedling crown, seedling leaf, immature inflorescence, floral bracts before anthesis, pistil before anthesis, anthers before anthesis, 3-5 DAP caryopsis, 22 DAP embryo, 22 DAP endosperm). The 61k wheat Affymetrix microarray data is obtained from PLEXdb and expression values are given in log2 units. Three replicates for each tissue are used. The significance differences in the gene expression level is estimated using one way ANOVA, followed by Duncan's multiple range test. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

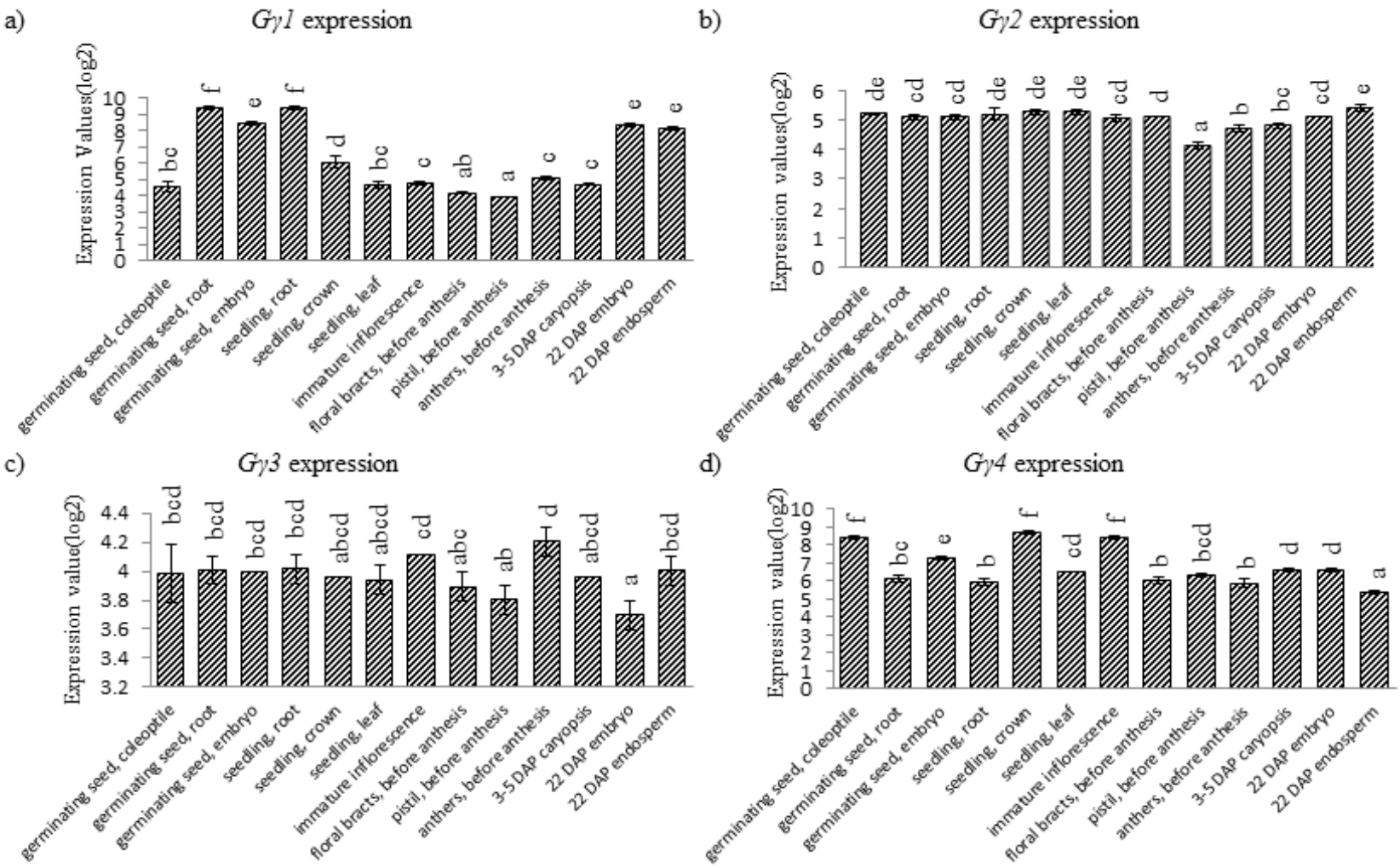

Figure 2.5. Tissue specific gene expression of Gγ's in *Triticum aestivum* by microarray. a) *Gγ1* b) *Gγ2* c) *Gγ3* and d) *Gγ4* were measured across a panel of thirteen different developmental stages (germinating seed coleoptile, germinating seed root, germinating seed embryo, seedling root, seedling crown, seedling leaf, immature inflorescence, floral bracts before anthesis, pistil before anthesis, anthers before anthesis, 3-5 DAP caryopsis, 22 DAP embryo, 22 DAP endosperm). The 61k wheat Affymetrix microarray data is obtained from PLEXdb and expression values are given in log2 units. Three replicates for each tissue are used. The significance differences in the gene expression level is estimated using one way ANOVA, followed by Duncan's multiple range test. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

2.4.7. Gene expression in response to osmotic, heat and combined stress

Gene expression analysis in response to osmotic, heat and combined osmotic and heat stress was analysed from the RNA-Seq datasets by Liu et al. 2015. The germinated seeds from TAM107 wheat cultivar were grown on filter paper in petri dishes under long day conditions with 22/18°C temperatures. Osmotic stress and heat stress were administered by subjecting seedlings to 20 % (m/V) of PEG-6000 or to 40°C temperature treatments, while combined stress was given by subjecting seedling to both 20 % PEG-6000 and 40°C temperature for 1 hr and 6 hr.

Most of the G protein gene families had the significant decreases or increases in the gene expression in response to osmotic stress, heat and combined heat and osmotic stresses in the wheat cultivar TAM107 under at least one stress treatment condition for at least one homeologous gene copy. Among the treatments, most of the significant increase or decrease in the gene expression of G protein genes were detected after 1hr or 6hr of heat and combined osmotic and heat stress treatment. The A homeologous copy of *GA1* had the significant decrease in mRNA levels after 1hr of heat and combined stress and, which was 0.06 and 0.08 fold respectively. The *Gβ*-B homolog showed more than 3 fold decrease in expression after 1hr of heat and combined stress treatment. The low level of 0.01 RPKM gene expressions for *Gγ1*-A was detected in control conditions; however after 1hr of osmotic stress treatment it was induced by 5.7 fold. The significant differences in the gene expression for *Gγ2* and *Gγ4* homeologs were detected after one or six hour of heat or combined osmotic and heat stress treatments. *Gγ2*-D showed a significant 2.17 fold induction of expression after 6hr of heat stress. The homeologous copies of *Gγ4* responded differently to one or more stress treatment conditions and significant decrease of 10 fold and 14.28 fold in the gene expression of –A and –D homeologs after 6hr of heat stress treatment were detected respectively, whereas *Gγ4*–B was most strongly repressed after 1hr of combined osmotic and heat stress, which led to a 0.08 fold decrease. *Gγ3* homeologs showed more than 4 fold decrease after 1 hr of heat and combined stress treatment. The details for the data are given in **Figure 2.6 and 2.7,** and fold change is given in **Table 2.2**.

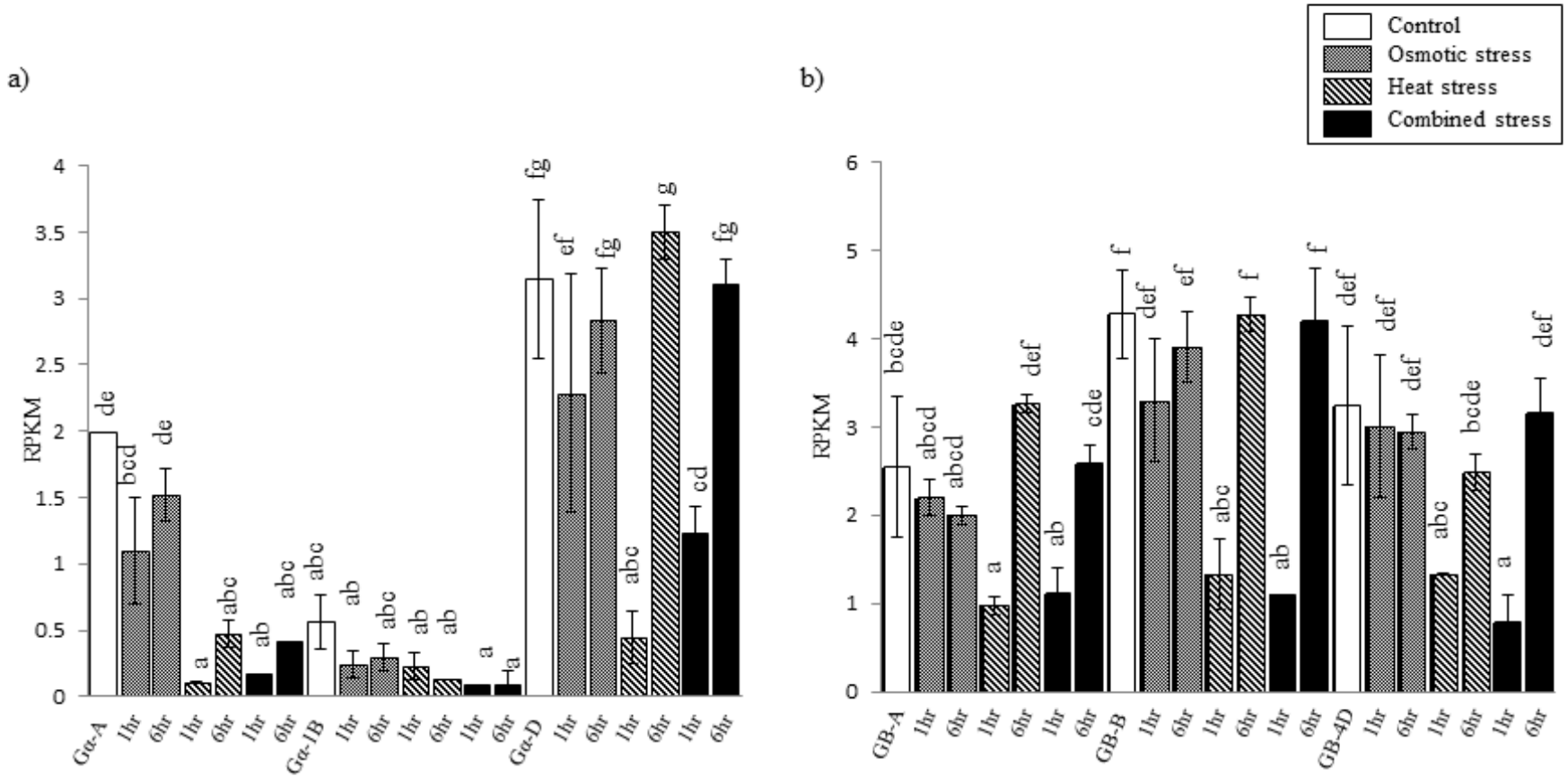

Figure 2.6. Expression analysis of *Gα* and *Gβ* in response to osmotic, heat and combined stress by RNA-Seq. The gene expression level of heterotrimeric G protein gene families in *Triticum aestivum* in response to short term osmotic, heat and combined osmotic and heat stress for 1hr and 6hrs. A, B and D homeologs for a) Gα and b) Gβ are given. The expression values are represented as reads per kilo base per million (RPKM). RPKM values are based on two replicates each. The significance of differences in the gene expression is estimated using one way ANOVA. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard error of the means.

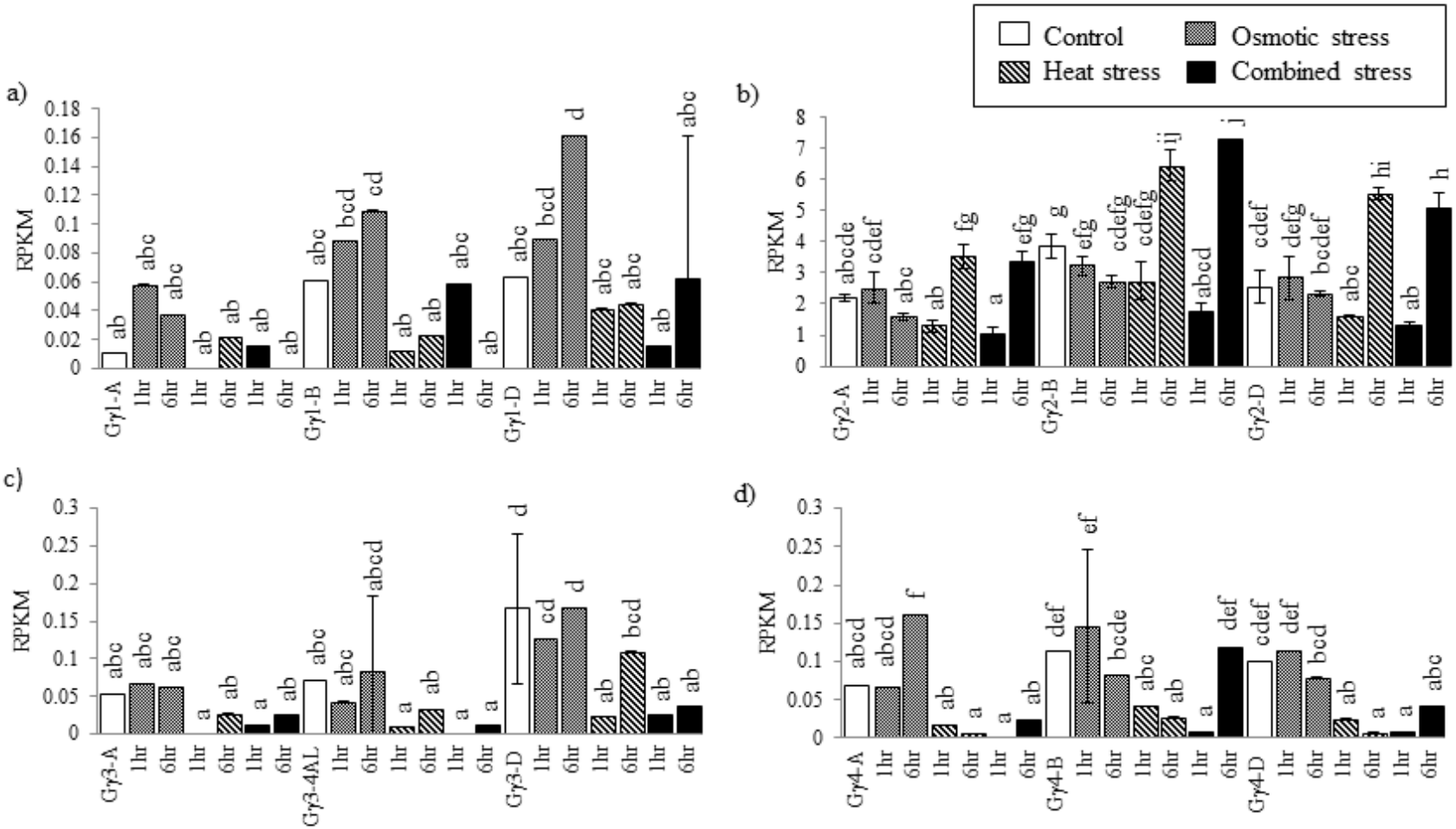

Figure 2.7. Expression analysis of *Gγ*'s in response to osmotic, heat and combined stress by RNA-Seq. The gene expression level of Gγ's in *Triticum aestivum* in response to short term osmotic, heat and combined osmotic and heat stress for after 1hr and 6hrs stress treatment is determined. Expression level for A, B and D homeologs of a) *Gγ1* b) *Gγ2* c) *Gγ3* and d) *Gγ4* are given. The expression values are represented as reads per kilo base per million (RPKM). RPKM values are based on the two replicates each. The significance of differences in the gene expression is estimated using one way ANOVA. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

Table 2.2. *T. aestivum* G protein gene family members expression and fold change in response to osmotic, heat and combined stress assayed by RNA-Seq

	Expression values in RPKM							Fold change					
Gene	CN	Osmotic stress		Heat stress		Combined		Osmotic stress		Heat stress		Combined	
		1hr	6hr	1hr	6hr	1hr	6hr	1hr	6hr	1hr	6hr	1hr	6hr
GA1-A	2.00	1.10	1.52	0.11	0.48	0.16	0.41	0.55	0.76	0.06	0.24	0.08	0.20
GA1-B	0.56	0.24	0.30	0.23	0.13	0.09	0.09	0.43	0.53	0.40	0.24	0.16	0.16
GA1-D	3.15	2.29	2.83	0.45	3.50	1.23	3.10	0.73	0.90	0.14	1.11	0.39	0.98
Gβ-A	2.55	2.20	2.00	0.98	3.26	1.11	2.59	0.87	0.79	0.38	1.28	0.44	1.02
Gβ-B	4.28	3.30	3.91	1.33	4.27	1.10	4.21	0.77	0.91	0.31	1.00	0.26	0.98
Gβ-D	3.24	3.01	2.95	1.34	2.49	0.79	3.16	0.93	0.91	0.41	0.77	0.24	0.97
Gγ1-A	0.01	0.06	0.04	0.00	0.02	0.02	0.00	5.70	3.67	0.00	2.12	1.51	0.00
Gγ1-B	0.06	0.09	0.11	0.01	0.02	0.06	0.00	1.45	1.79	0.20	0.37	0.96	0.00
Gγ1-D	0.06	0.09	0.16	0.04	0.04	0.02	0.06	1.42	2.54	0.65	0.71	0.24	0.97
Gγ2-A	2.19	2.50	1.59	1.30	3.51	1.05	3.38	1.14	0.72	0.59	1.60	0.48	1.54
Gγ2-B	3.85	3.23	2.73	2.73	6.43	1.75	7.31	0.84	0.71	0.71	1.67	0.45	1.90
Gγ2-D	2.55	2.85	2.33	1.62	5.53	1.32	5.09	1.11	0.91	0.63	2.17	0.52	1.99
Gγ3-A	0.05	0.07	0.06	0.00	0.03	0.01	0.02	1.25	1.17	0.00	0.51	0.24	0.47
Gγ3-AL	0.07	0.04	0.08	0.01	0.03	0.00	0.01	0.60	1.18	0.13	0.46	0.00	0.15
Gγ3-D	0.17	0.13	0.17	0.02	0.11	0.02	0.04	0.76	1.01	0.14	0.66	0.15	0.21
Gγ4-A	0.07	0.07	0.16	0.02	0.01	0.00	0.02	0.99	2.38	0.25	0.10	0.00	0.36
Gγ4-B	0.11	0.15	0.08	0.04	0.03	0.01	0.12	1.30	0.73	0.37	0.24	0.08	1.05
Gγ4-D	0.10	0.11	0.08	0.02	0.01	0.01	0.04	1.15	0.80	0.25	0.07	0.09	0.42

Note: The germinated seeds from TAM107 wheat cultivar were grown on filter paper in petri dishes under long day conditions. Osmotic stress was given by 20% PEG 6000 and heat stress by 40°C for 1hr and 6hr. Combined stress was given by both 20% PEG 6000 and by 40°C. The values for expression are given in Reads per kilobase per million (RPKM). CN is control treatment.

The Affymetrix microarray analysis of two *T. turgidum* cultivars, sp. *durum*, Cappeli with high water use efficiency (WUE) and Ofanto with low water use efficiency respectively, were given drought, heat and combined stress at booting stage of plant, and the flag leaf tissues were used for transcriptome analysis (Aprile et al., 2013) The microarray analysis showed that most of the G protein genes in Ofanto cultivar had similar gene expression with Cappeli cultivar. Differences of less than two folds were detected in the gene expression of *Gγ2* were detected in two cultivars after combined stress treatment. The details for the microarray data are given in **Figure 2.8** and **Supplementary Table S2.12.**

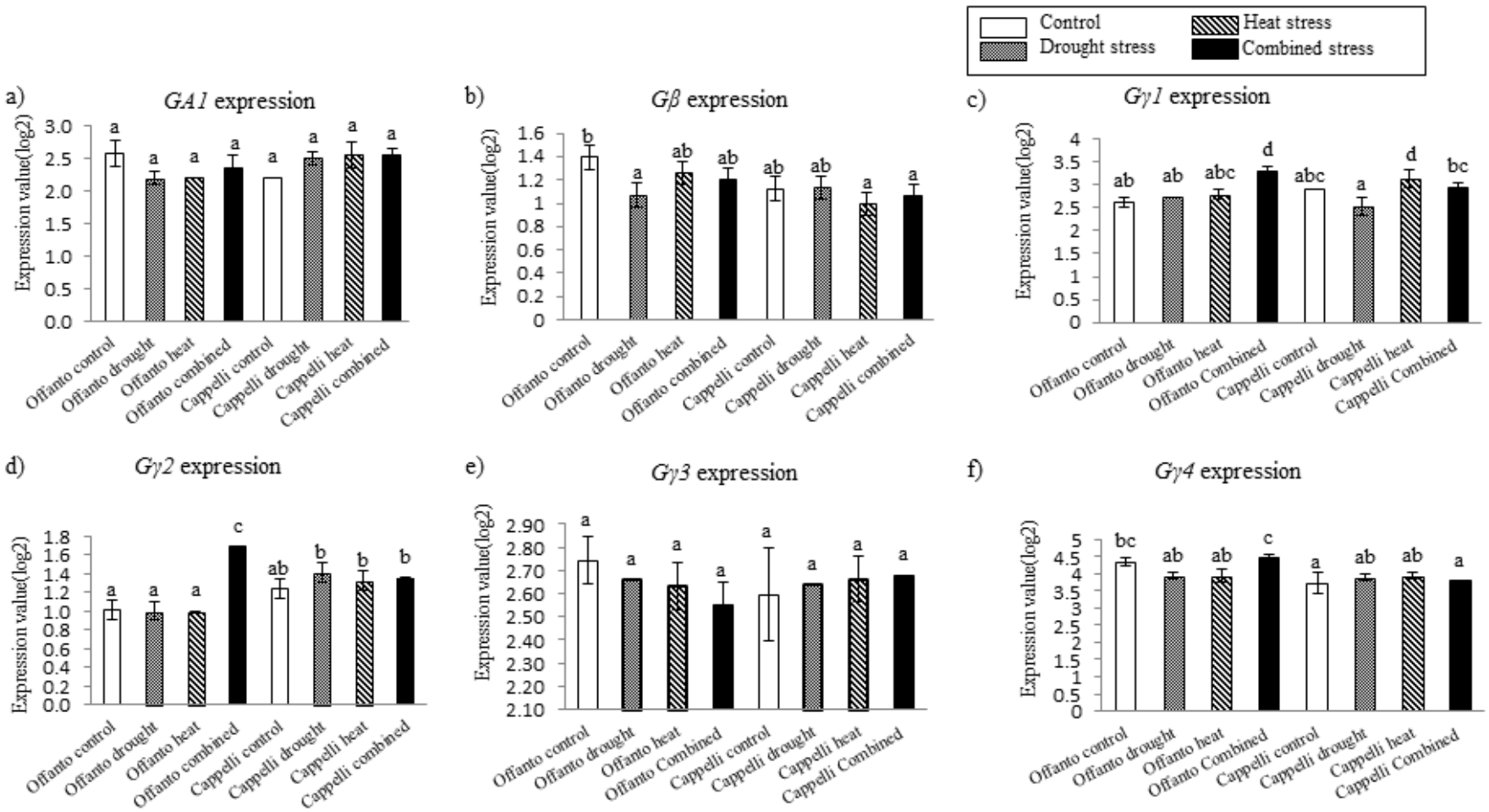

Figure 2.8. Expression analysis G protein gene families in response to drought, heat and combined stress by microarray. The gene expression analysis of *T. aestivum* heterotrimeric G protein genes a) *GA1* b) *Gβ* c) *Gγ1* d) *Gγ2* e) *Gγ3* d) and f) *Gγ4* was measured in response to the drought, heat and combined stress in two *T. durum* cultivars Ofanto and Cappeli, with lower and higher water use efficiency respectively using 61k wheat Affymetrix microarray. Expression values are given in log2 units. Three replicates for each treatments are used. The significance differences in the gene expression level is estimated using one way ANOVA, followed by Duncan's multiple range test. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

2.4.8. Gene expression in response to cold stress

The changes in the gene expression of G protein gene families in *T. aestivum* cv Manitou was analysed from the datasets by Li et al. 2015. Plants were grown in soil for two weeks at 23°C and then were moved to 4°C for two weeks for cold stress treatment, while the control plants were kept at 23°C for two weeks. Only *GA1*-A and *Gγ1*-D showed the significant differences in gene expression in response to cold stress. *GA1*-A was down regulated 0.27 fold after cold stress treatment, whereas *Gγ1*-D was upregulated by 4.73 fold. The details for gene expression analysis in response to stress treatments are given in **Figure 2.9** and **Table 2.3.**

The 61k wheat Affymetrix microarray transcriptome data for cold stress treatment was analyzed in two spring habit (spring Manitou and spring Norstar) and two winter habit (winter Manitou and winter Norstar) cultivars, in which locus for the vernalization gene, *Vrn-A1* was swapped between the parent cultivars winter Norstar and spring Manitou with near isogenic lines (NIL) of spring Norstar and winter Manitou. The genotype spring Manitou had tender spring habit and the winter Norstar had cold hardy winter habit. The cold acclimation treatment to plants was given at 6°C for 2, 14, 21, 35, 42, 56 and 70 days and transcriptome analysis was carried out using the three leaf stage crown tissues (Laudencia-Chingcuanco et al., 2011). Very small but statistically significant differences in gene expression of *Gβ* and *Gγ4* for spring and winter habit cultivars upon cold stress treatment were detected, however these changes were less than two folds. After cold stress treatment for 56 and 70 days spring Manitou cultivar had greater reduction in expression of *Gβ* than winter Manitou cultivars. Similarly, small but statistically significant differences in the gene expression of *Gγ4* were detected between spring Norstar and winter Norstar **(Figure 2.10 and Supplementary Table S2.13)** as well as between spring Manitou and winter Norstar cultivars in all the treatment after 14 days of cold acclimation and higher induction of *Gγ4* was detected in spring cultivars. These results suggest that in winter habit and spring habit cultivars *Gβ* and *Gγ4* respond differently and *Vrn-A1* gene affects the expression of these genes. The details for the microarray data analysis in response to cold stress in spring and winter habit cultivars are given in **Figure 2.10 and Supplementary Table S2.13.**

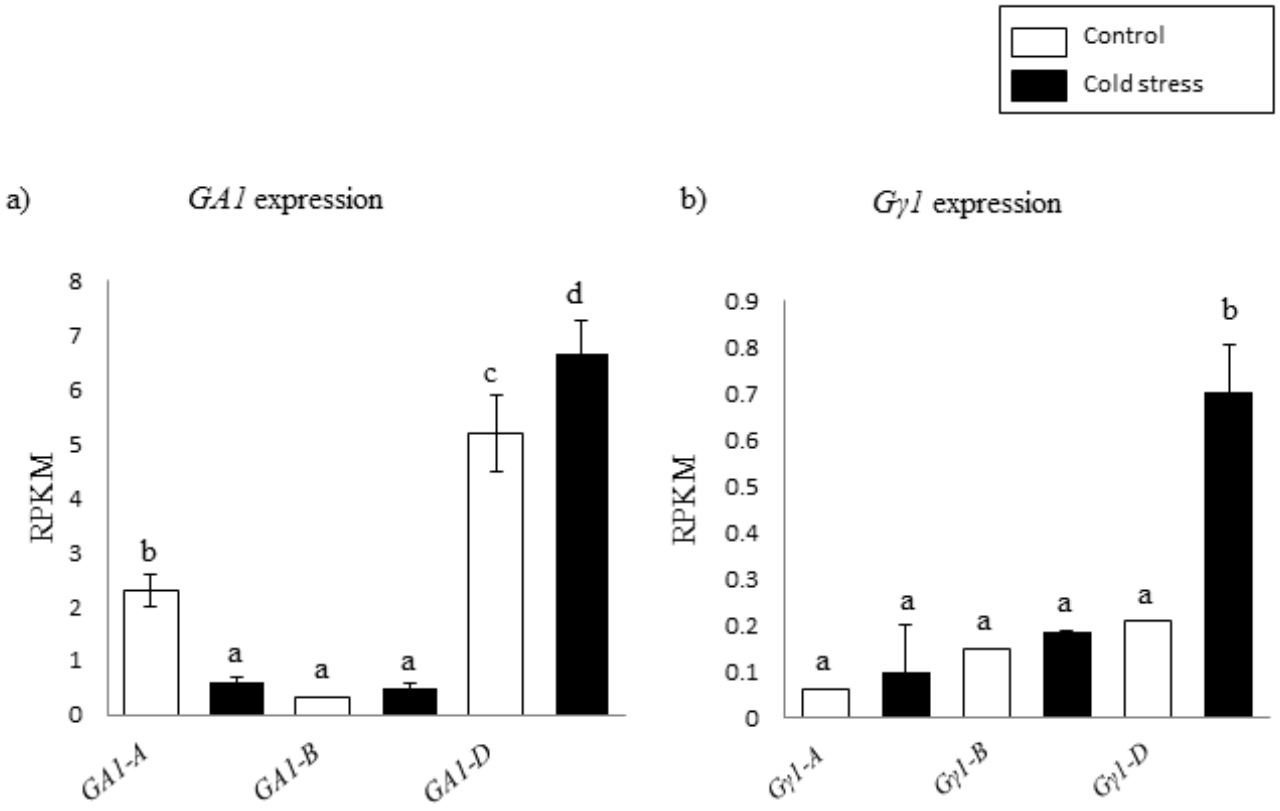

Figure 2.9. Expression analysis in response to the cold stress by RNA-Seq. The expression for *T. aestivum* heterotrimeric G protein gene a) *GA1* and b) *Gγ1* is given. The expression values are represented as reads per kilo base per million (RPKM). RPKM values are based on three replicates each. The significance of differences in the gene expression is estimated using one way ANOVA. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

Table 2.3. *T. aestivum* G protein gene family members expression in response to cold stress assayed RNA-Seq

	Expression RPKM		
Gene	Control	Cold stress	Fold change
	23°C	4°C	
GA1-A	2.30	0.61	0.27
GA1-B	0.34	0.49	1.46
GA1-D	5.19	6.65	1.28
Gβ-A	1.83	1.25	0.68
Gβ-B	3.50	3.43	0.98
Gβ-D	2.76	3.47	1.26
Gγ1-A	0.06	0.10	1.63
Gγ1-B	0.21	0.19	0.90
Gγ1-D	0.15	0.70	4.73
Gγ2-A	0.00	0.00	0.00
Gγ2-B	6.14	5.91	0.96
Gγ2-D	10.64	7.12	0.67
Gγ3-A	0.11	0.00	0.00
Gγ3-AL	0.32	0.08	0.25
Gγ3-D	0.14	0.03	0.20
Gγ4-A	0.02	0.01	0.82
Gγ4-B	0.05	0.04	0.82
Gγ4-D	0.05	0.06	1.32

Note: *T. aestivum* cv Manitou plants were grown in soil for two weeks at 23°C and then were moved for two weeks at 4°C temperature for cold stress treatment. Control plants were kept at 23°C for two weeks. ND indicates not determined. Expression values are given in Reads per kilo base per million (RPKM).

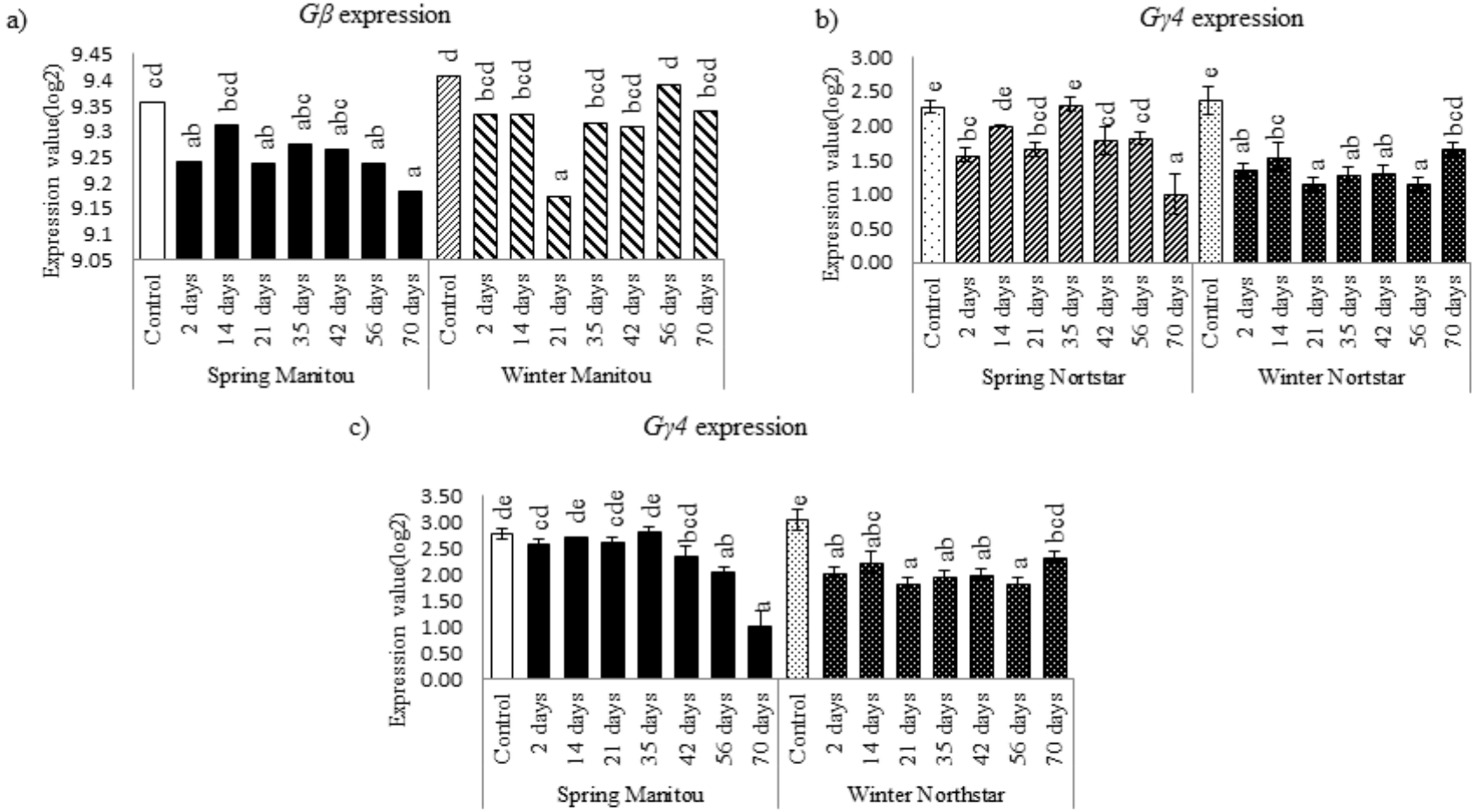

Figure 2.10. Expression analysis in response to cold stress assayed by microarray. The expression for a) *Gβ*, b) and c) *Gγ4* in spring and winter habit cultivars was measured in response to the cold stress by 61k Affymetrix microarray. The 61k wheat Affymetrix microarray data is obtained from PLEXdb and expression values are given in log2 units. Three replicates for each treatments are used. The significance differences in the gene expression level is estimated using one way ANOVA, followed by Duncan's multiple range test. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

2.4.9. Gene expression analysis in response to *Fusarium graminearum* infection

The difference in the gene expression of G protein genes, after inoculation of *F. graminearum* spore suspension in the two wheat cultivars NIL38, which is homozygous for Fusarium resistance alleles at QTL, Fhb1 and Qhfs.ifa-5A, and NIL51, which is homozygous for susceptible alleles at same QTL, were analysed after 24 and 48 hrs after inoculation. Only *Gγ1*-D homeolog had the significant differences in the gene expression levels in resistant NIL38 and susceptible NIL51 cultivars after 48hr of *F. graminearum* spore suspension inoculation. *Gγ1*-D was upregulated in resistant NIL38 *F. graminearum* spore suspension inoculation by 6.43 fold, whereas in NIL51 the increase in gene expression was found to be 1.90 fold. These results showed that *Gγ1*-D respond differently in resistant NIL38 and susceptible NIL51 cultivars in response to *F. graminearum*. The details for the gene expression analysis upon *F. graminearum* infection are given in **Figure 2.11** and **Table 2.4.**

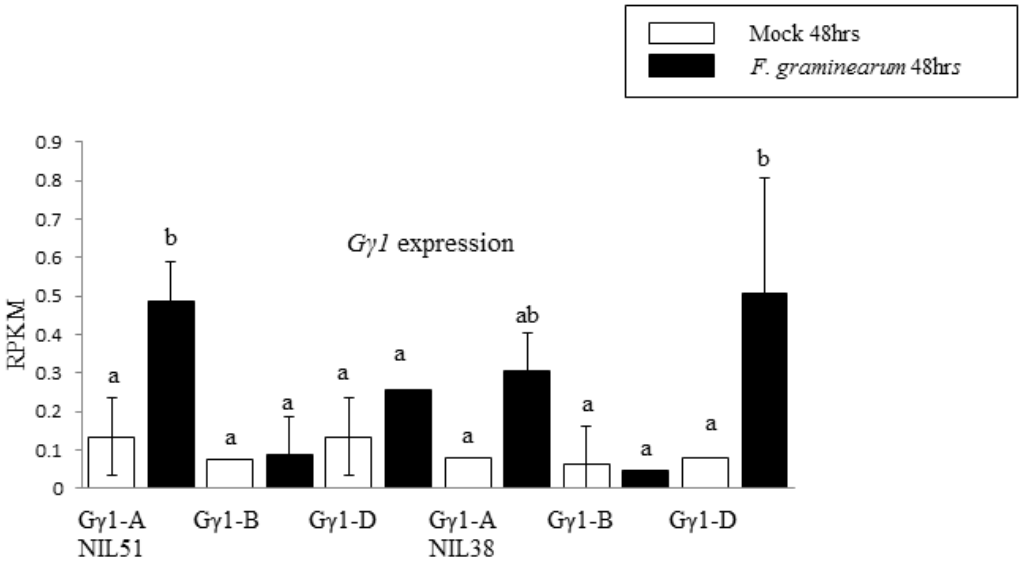

Figure 2.11. Gene expression analysis in response *F. graminearum*. The gene expression analysis of *T. aestivum Gγ1* in response to the *F. graminearum* spore suspension inoculation in disease susceptible line NIL51 and disease resistant line NIL38 after 48hrs. The expression values are represented as reads per kilo base per million (RPKM). RPKM values are based on three replicates each. The significance of differences in the gene expression is estimated using one way ANOVA. The different letters on the each bar indicates the rankings assigned by Duncan's test ($p \leq 0.05$) and the error bars represent standard errors of the means.

Table 2.4. Fold change for *T. aestivum* G protein genes response to *F. graminearum* inoculation by RNA-Seq

Table 2.4-A. Fold change in NIL 51 and NIL 38 after 24 hr of inoculation

Gene	24hrs Mock RPKM		24hrs *Fg* RPKM		Fold change 24hrs	
	NIL51	NIL38	NIL51	NIL38	NIL51	NIL38
GA1-A	2.70	2.39	2.59	3.08	0.96	1.29
GA1-B	1.72	1.79	1.29	1.44	0.75	0.80
GA1-D	3.52	3.52	3.50	4.61	0.99	1.31
Gβ-A	4.30	3.95	4.00	3.90	0.93	0.99
Gβ-B	6.01	7.19	5.27	6.14	0.88	0.85
Gβ-D	4.53	5.08	5.55	4.45	1.23	0.88
Gγ1-A	0.19	0.20	0.14	0.34	0.73	1.71
Gγ1-B	0.10	0.11	0.12	0.10	1.23	0.92
Gγ1-D	0.15	0.08	0.18	0.18	1.22	2.16
Gγ2-A	1.42	1.62	1.52	1.26	1.07	0.78
Gγ2-B	2.46	1.90	2.57	2.10	1.04	1.11
Gγ2-D	2.06	1.97	1.93	2.02	0.94	1.03
Gγ3-A	0.09	0.09	0.20	0.13	2.22	1.44
Gγ3-AL	1.95	0.14	1.89	0.22	0.97	1.56
Gγ3-D	0.93	1.30	1.61	1.41	1.73	1.09
Gγ4-A	0.98	0.75	0.74	0.64	0.75	0.85
Gγ4-B	0.91	0.90	0.71	0.63	0.78	0.70
Gγ4-D	1.54	1.23	1.31	0.92	0.85	0.75

Table 2.4-B. Fold change in NIL 51 and NIL 38 after 48 hr of inoculation

Gene	48hrs Mock RPKM		48hrs *Fg* RPKM		Fold change 48hrs	
	NIL51	NIL38	NIL51	NIL38	NIL51	NIL38
GA1-A	1.86	2.65	2.08	3.43	1.12	1.30
GA1-B	2.15	1.33	1.92	1.30	0.89	0.98
GA1-D	4.48	4.48	4.75	4.08	1.06	0.91
Gβ-A	4.36	4.29	4.31	4.55	0.99	1.06
Gβ-B	7.19	6.84	6.74	6.94	0.94	1.01
Gβ-D	5.60	5.13	4.95	4.98	0.88	0.97
Gγ1-A	0.13	0.08	0.49	0.31	3.64	3.85
Gγ1-B	0.08	0.06	0.09	0.05	1.13	0.75
Gγ1-D	0.13	0.08	0.26	0.51	1.90	6.43
Gγ2-A	1.67	1.82	1.82	1.63	1.09	0.90
Gγ2-B	1.88	2.03	2.21	3.53	1.18	1.74
Gγ2-D	2.37	2.03	2.83	2.29	1.19	1.13
Gγ3-A	0.14	1.45	0.09	2.06	0.63	1.41
Gγ3-AL	2.11	2.06	2.67	2.26	1.27	1.10
Gγ3-D	0.94	2.43	1.73	2.43	1.85	1.00
Gγ4-A	0.80	0.69	0.53	0.42	0.67	0.61
Gγ4-B	1.03	0.73	0.53	0.49	0.51	0.68
Gγ4-D	1.54	0.99	1.00	0.97	0.65	0.98

Note: Gene expression analysis in response to *F. graminearum* by inoculation in spikelets of resistant NIL38 and susceptible cultivar NIL51, analysed after 24hr and 48 hr of inoculation, mock are the controls at respective times , *fg* denotes *F. graminearum* inoculation treatments Gene expression given RPKM

2.5. Discussion

2.5.1. G protein gene families in *Triticum aestivum* and monocots

The G protein gene families in *T. aestivum* are comprised of single GA1, single Gβ and four Gγ's, per haploid genome, and with three homeologous copies of A, B and D homologs for each gene. The higher degree of similarity, approximately 97 %, in the nucleotide coding regions between the homeologous groups of genes of *T. aestivum* is similar to that reported for alpha tubulin, caleosins and Early Salt Induced 3 (Esi3) gene families (Ridha Farajalla and Gulick, 2007; Khalil et al., 2014 and Brunetti et al., 2018). Surprisingly, three homeologous copies of *GA1* were represented on chromosome 7AS, 1BL and 7DS respectively. Large translocations between 7B and 1B have not been described by Devos et al. 1995, though the intergenomic comparison of *Ae. tauschii* and *T. aestivum* did identify numerous small translocations between chromosomes in these species (Dvorak et al., 2018). The B copy of *GA1* appears to be the result of a single gene translocation, genes flanking Gα on chromosome 1B, have homeologs that map to similar relative positions on chromosomes 1A and 1D.

The three gene sequence accessions for Gα subunits in *T. aestivum* had been represented in GenBank NCBI NR database. Ta*GA1* (AB090158.2), Ta*GA2* (AB090159.1) and Ta*GA3* (HQ020506.1) appear to represent a single gene since they have large portions of the sequences with 100% sequence identity. HQ020506.1 corresponds to the B genome homeolog of Gα, and of the three sequence, only it is supported by the cDNA sequences in the TSA and EST databases (Khalil et al., 2011) the other variants (Hossain et al., 2003), may represent allelic or splice variants or sequencing errors. We refer this gene as Ta*GA1* in our study.

The *Gβ* homeologs shared more than 98.5% similarity in the nucleotide coding regions and encode the protein of lengths 380aa. The similar protein lengths of 380aa were found for Gβ's in Arabidopsis and monocot species in this study.

The diversity of protein sequences among the Gγ's found in this study had also been observed in other species, where the number of Gγ's varied between three to five per diploid genome in the species Arabidopsis and soybean respectively. In total four Gγ's in *T. aestivum* were identified, of which most of *Gγ*'s were represented on same short or long arm of A, B or D homeologous

chromosomes at IWGSC database, except for *Gγ3*. Nearly all the sequences at IWGSC for *Gγ*'s had FL coverage and were in agreement with the TSA database; however, some of the sequences including *Gγ1*-B and homeologs of *Gγ3* were not represented in EST databases. Most of the other *Gγ* sequences could be confirmed by FL or partial length coverage with sequences in the EST database at NCBI with representation of three to six EST sequences for each gene. *Gγ-3*, *-4* and *-5* are categorised as type C, which are the largest among the gene family and have conserved random cysteine residues in C-terminal regions. The tblastn search with Gγ3 protein sequence at NR database showed that it hits on the keratin associated protein 5 in most of the species; however in rice also the *Gγ3* had been shown to hit for the same protein, this is likely due to the mis-annotation of *Gγ3* as keratin associated proteins at NR database. The B homeolog of *Gγ3* was located on long arm of 4A chromosome instead of 7BS and this was due to the reciprocal translocation between the 7BS and 4AL chromosomes, which had been reported previously. The genetic map study of bread wheat had shown that 4AL chromosome included a 52cM region from 7BS chromosome, whereas 36cM region of 5AL had also been found on the long arm of 4A. Studies with RFLP markers and chromosome paring showed that these rearrangements and the translocation had occurred in the tetraploid wheat progenitor before *T. aestivum*'s hexaploidy event (Devos et al., 1995).

The validation of the Gγ's in *T. aestivum* as proteins that interact with Gβ were carried out using BiFC. The *in vivo* protein–protein interaction for the Gγ1 and Gγ2 subunits were both shown to interact with Gβ and the interaction was localized to the plasma membrane. Gβ, Gγ1 and Gγ2 were also shown to localize to plasma membrane when they were expressed as proteins fused to full-length GFP.

Most of the monocot species in this study had four *Gγ*'s like Triticum, except for the *S. cereale*, which only showed three *Gγ*'s. The *S. cereale Gγ3* was not detected among the sequences of TSA databases at NCBI and from the whole genome draft assembly (https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_rye.cgi). In rice, five *Gγ*'s had been identified previously (Trusov et al., 2012) and the search with rice *Gγ*'s in the genome of *S. bicolor* and *Z. mays* showed that these species also had five *Gγ*'s each.

2.5.2. Conserved regions and phylogenetic analysis

The regions identified previously in the G protein gene families as characteristics features of genes were found to be conserved in *T. aestivum* and other monocot species and Arabidopsis. The A and D homologs of *GA1* had 12 exons each, which were similar gene structure found in *Ae. tauschii,* the progenitor to the D genome of *T. aestivum*, whereas the *GA1*-B homeolog located on chromosome 1BL contained 13 exons **(Supplementary Table S2.8**). Sequence comparison showed that the 11th exon had an insertion of an intron which split it into two exons. The last 13th exon in the *GA1*-B had 17nt duplication relative to the A and D gene copies, which caused a frame shift and a shorting of the open reading frame of 62 nt, which was responsible short length of *GA1*-B proteins (367 aa) relative to proteins encoded by the -A and -D homeologs which had 385 and 382 aa respectively. This mutation present in the *GA1*-B appears to be an allelic variant that is present in the cultivar Chinese Spring and cultivar Kenong 199 (Dong et al., 2019), however EST sequences for this gene from *T. aestivum* cultivars Atlas66 and Cranbrook (CJ568330.1 and HX178860.1) did not have the duplication. The C terminal region of *Ta*-Gα-7A was shown to interact with central hydrophilic region of COLD1, a gene shown to regulate the plant height (Dong et al., 2019). However, *T. aestivum GA1*-B from Kenong 199 which has 17 nt duplication in C terminal end did not interact with COLD1. This suggests that C terminal region of *T. aestivum* GA1 is critical for the physical interaction, at least with COLD1.

The phylogenetic analysis using protein sequences for G protein gene families in *T. aestivum*, Arabidopsis and other monocot species showed that more closely related Triticeae tribe species like *T. aestivum, A. tauschii, H. vulgare* and *S. cereale* were grouped together (**Supplementary Figure S2.5 and S2.6**). Arabidopsis Gγ1 and Gγ2 have prenylation –CaaX motif, whereas in monocots only Gγ1 has this motif, Gγ2 in monocots are comprised of conserved non-prenylated motif -KGDSFA or -KGSDFS. This suggests that the evolution non-prenylated *Gγ2* is the gene modification event which had been occurred after the divergence of dicot and monocot lineages. Similarly, Arabidopsis *Gγ3* belongs to Type C, while *T. aestivum* and other monocot species *Gγ3*, *-4* and *-5* belong to type C. This suggests that gene duplication in *Gγ*'s events occurred after the divergence of the dicot and monocots lineage, primarily among the type C *Gγ*'s, which gave rise to Gγ4 and Gγ5 in monocots. It can also be inferred that type C *Gγ*'s are the most recently evolved *Gγ*'s. *O. sativa*, *Z. mays* and *S. bicolor* have five *Gγ*'s each, while Triticeae tribe species has three to four. This suggest that either recent duplication events in *O. sativa*, *Z. mays* and *S. bicolor* lineages occurred after speciation which gave rise to *Gγ5* or recent deletion events

occurred in Triticeae tribe after speciation that resulted in the loss of *Gγ5* (**Supplementary Figure S2.7 and S2.8**).

2.5.3. Gene expression analysis

The diverse expression pattern in the different tissue types and developmental stages were analyzed for G protein gene families in *T. aestivum*. The similar pattern of expression like the UTR regions were found for the Ta- GA1 and Ta-Gβ and Ta-Gγ2 cDNA sequences when coding regions were used except that it gave large number of hits. Hence, we used the 3' UTR regions for the analysis of these genes. The high level of gene expression for homeologs of *GA1*, *Gβ* and *Gγ2* and low level of gene expression for homeologs *Gγ2, Gγ3 and Gγ4* were detected in most of the tissues analysed in Azhurnaya spring wheat. The higher level of gene expression within the homeologous copies of genes may be due to the reason that one of the progenitor species contributed some element of selective advantage, possibly through the regulation of gene expression of specific gene family members, rather than variation is protein structure .The similar levels in the gene expression within homeologous group were also reported for other gene families like *Esi3* (*Early Salt Induced 3*) in *T. aestivum* (Brunetti et al., 2018) though the large difference in expression among homeologous gene family member for *GA1* and *Gγ1* was exceptions to this tendency. The tissue specific expression analysed by RNA-Seq and 61k wheat microarray were in agreement with each other. The most striking result from the transcriptome dataset analysed in Azhurnya spring wheat was the higher level of expression of *Gβ* in the stigma and ovary tissues compared to other seventy one tissue types, which suggest that these genes may have a role in the reproductive organ of plants especially in the stigma and ovary development. *Gγ3* was the least expressed gene in tissues and low level of expressions for *Gγ4* were also detected in most of the tissues.

Most of the G protein gene family members in *T. aestivum* showed more than two fold change in the expression in response to one or another stress conditions. The homeologs *GA1*-A and G*γ1*-D showed significant differences in the gene expression in more than one stress conditions by RNA-Seq. For example, *GA1*-A was down regulated in response to heat and cold stress treatments and *Gγ1*-D was upregulated in response to cold stress treatment and *F. graminearum* inoculation in resistant cultivar NIL38, suggesting that these genes may contribute in stress

tolerance to more than one stress conditions. *GA1* and *Gβ* homeologs showed the significant changes in gene expression in both heat and combined osmotic and heat stress treatments.

GA1 in *Triticum aestivum* did not show higher fold changes in drought and cold stress. However, GA1 in *T. aestivum*, previously called as GA3 (Khalil et al., 2011) is known to interact with caleosin 3 (Clo3), also known as RD20, which is in Arabidopsis is known to induce by ABA, drought and salt stress and positively regulates drought tolerance (Aubert et al., 2011). The rice GA1 (RGA1) is known to interact with COLD1 and contribute to the cold tolerance in japonica rice (Ma et al., 2015). These studies suggest that GA1 subunits in plant species can also act as regulatory proteins, which act through different interacting partners and contribute to stress tolerance.

Most of the RNA-Seq transcriptome analysis results were in agreement with the 61K wheat Affymetrix microarray data analysed. Some notable interesting results in the microarray data analysis were the higher induction of *Gγ2* in the low WUE cultivar Ofanto compared to high WUE cultivar Cappeli under combined osmotic and heat stress suggesting that *Gγ2* may be associated with WUE in the *T. aestivum*. Other interesting results were the differences in the expression of *Gβ* and *Gγ4* genes in spring and winter habit cultivars in which vernalization gene *Vrn-A1* was swapped in the cultivars. This indicate that *Vrn-A1* regulates the gene expression *Gβ* and *Gγ4* in spring and winter habit and possibly act upstream of *Gβ* and *Gγ4* in the pathway.

The expression analysis in response to stress conditions showed that G protein gene families in *T. aestivum* had responded to abiotic stress conditions including cold acclimation, osmotic stress, heat stress and combined osmotic and heat stress as well as biotic stress like *F. graminearum* infections. It will be interesting to know if these genes have functional roles in these stress responses, which needs to be validated further.

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                          *  *                                                  G1
T.aestivum_GA1-A          MGSSCSRPHSVNEADAADNTRSADIDRRILHETKADQHIHKLLLLGAGESGKSTIFKQIK   60
T.aestivum_GA1-B          MGSSCSRPHSVNEAEAADNTRSADIDRRILQETKADQHVHKLLLLGAGESGKSTIFKQIK   60
T.aestivum_GA1-D          MGSSCSRPHSVNEADAADNTRSADIDRRILQETKADQHIHKLLLLGAGESGKSTIFKQIK   60
A.tauschii_Gα             MGSSCSRPHSVNEADAADNTRSADIDRRILQETKADQHIHKLLLLGAGESGKSTIFKQIK   60
S.cereale_Gα              MGSSCSRPHSVNEAEAADNRRSADIDRRILQETKADQHVHKLLLLGAGESGKSTIFKQIK   60
H.vulgare_Gα              MGSSCSRPHSVNEAEAAGNTRSADIDRRILHETKADQHIHKLLLLGAGESGKSTIFKQIK   60
B.distachyon_Gα           MGSSCSRPH-LNEAEAAENGKSAEIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIK   59
O.sativa_Gα               MGSSCSRSHSLSEAETTKNAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIK   60
S.italica_Gα              MGSSCSRHHSLNEAEAAENAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIK   60
S.bicolor_Gα              MGSSCSRSHSLDETEAAENAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIK   60
Z.mays_Gα                 MGSSCSRSHSFDEAEAAENAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIK   60
A.thaliana_Gα             MGLLCSRSRHHTE-DTDENTQAAEIERRIEQEAKAEKHIRKLLLLGAGESGKSTIFKQIK   59

T.aestivum_GA1-A          LLFRTGFDEAELKGYTPVIHANVFQTIKILYDGAKELAQVESESSKYVMLPDNQEIGEKL   120
T.aestivum GA1-B          LLFRTGFDEAELKGYMPVIHANVFQTIKILYDGAKELAQLETESSKHVISPDNQEIGEKL   120
T.aestivum_GA1-D          LLFRTGFDEAELKGYTPVIHANVFQTIKILYDGAKELAQVEPESSKYVILPDNQEIGEKL   120
A.tauschii_Gα             LLFRTGFDEAELKGYTPVIHANVFQTIKILYDGAKELAQVEPESSKYVILPDNQEIGEKL   120
S.cereale_Gα              LLFRTGFDEAELKGYTPVIHANVFQTIKILYDGAKELAQMETESSKHVISPDNQEIGEKL   120
H.vulgare_Gα              LLFRTGFDEAELKGYTPVIHANVYQTIKILYDGAKELAQVELESSKYVISSDNQEIGEKL   120
B.distachyon_Gα           LLFQTGFDEAELRSYISVIHANVYQTIKILYDGAKELAQVEPESSKYVISPDNQEIGEKI   119
O.sativa_Gα               LLFQTGFDEAELRSYTSVIHANVYQTIKILYEGAKELSQVESDSSKYVISPDNQEIGEKL   120
S.italica_Gα              LLFQTGFDEAELRSYTSVIHANVYQTIKILYDGAKELAQVEPDSSKYVLSPDNQEIGEKL   120
S.bicolor_Gα              LLFQTGFDEAELKSYTSVIHANMYQTIKILYEGAKELAQVEPDSSKYVLSPDSQEIGEKL   120
Z.mays_Gα                 LLFQTGFDEAELRSYTSVIHANVYQTIKILYEGAKELAQVEPDSSKYVLSPDNQEIGEKL   120
A.thaliana_Gα             LLFQTGFDEGELKSYVPVIHANVYQTIKLLHDGTKEFAQNETDSAKYMLSSESIAIGEKL   119

T.aestivum_GA1-A          SEIGGRLDYPSLNKELVQDVRKLWEDQAIQETYSCGSVLQVPDCAHYFMDNLDRLAEADY   180
T.aestivum_GA1-B          SEIGGRLDYPLLNKELVQDVRKLWEDSAIQETYSCGSVLQVPDCAHYFMENLDRLAEPDY   180
T.aestivum_GA1-D          SEIGGRLDYPLLNKELVQDVRKLWEDQAIQETYSCGSVLQVPDCAHYFMDNLDRLAEADY   180
S.cereale_Gα              SEIGGRLDYPLLNKELVQDVRKLWEDPAIQETYSCGSVLQVPDCAHYFMENLDRLAEPDY   180
A.tauschii_Gα             SEIGGRLDYPSLNKELVQDVRKLWEDQAIQETYSCGSVLQVPDCAHYFMDNLDRLAEADY   180
H.vulgare_Gα              SEIGGRLDYPLLNKELVQDVRKLWEDPAIQETYSCGSVLQVPDCAHYFMENLDRLAEADY   180
B.distachyon Gα           SEIGGRLDYPLLCEELVHDIRKLWEDPAIQETYSRGSILQVPDCAQYFMENLDRLAEADY   179
O.sativa_Gα               SDIDGRLDYPLLNKELVLDVKRLWQDPAIQETYLRGSILQLPDCAQYFMENLDRLAEAGY   180
S.italica_Gα              SEIGAKLDYPLLNKELVQDVRKLWQDPAIQETYSRGSILQVPDCAQYFMSNLDRLAEVDY   180
S.bicolor_Gα              SEIGVRLDYPSLNKERVQDVRKLWQDPAIQETYSRGSILQVPDCAQYFMENLDRLSEVDY   180
Z.mays_Gα                 SEIGARLEYPSLNKERVQDVRKLWQDPAIQETYSRGSILQVPDCAQYFMENLDKLSEEDY   180
A.thaliana Gα             SEIGGRLDYPRLTKDIAEGIETLWKDPAIQETCARGNELQVPDCTKYLMENLKRLSDINY   179
                               G2                        G3
T.aestivum_GA1-A          VPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAV   240
T.aestivum_GA1-B          IPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAV   240
T.aestivum_GA1-D          VPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAV   240
A.tauschii_Gα             VPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAV   240
S.cereale Gα              VPTKEDVLHARVRTNGVVEIQFSP-GESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAV   239
H.vulgare_Gα              VPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAV   240
B.distachyon_Gα           VPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEIYRLYDVGGQRNERRKWIHLFEGVDAV   239
O.sativa_Gα               VPTKEDVLYARVRTNGVVQIQFSPVGENKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAV   240
S.italica_Gα              VPTKEDVLHARVRTNGVVETQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAV   240
S.bicolor_Gα              VPTKEDVLHARVRTNGVVETQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAV   240
Z.mays Gα                 VPTKEDVLHARVRTNGVVETQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAV   240
A.thaliana_Gα             IPTKEDVLYARVRTTGVVEIQFSPVGENKKSGEVYRLFDVGGQRNERRKWIHLFEGVTAV   239
                                       Switch I                  Switch II
T.aestivum_GA1-A          IFCAAISEYDQLLFEDETQNRMMETKELFDWVLKQTCFEKTSFMLFLNKFDIFERKIQKV   300
T.aestivum_GA1-B          IFCAAISEYDQLLFEDETQNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
T.aestivum GA1-D          IFCAAISEYDQLLFEDETQNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
A.tauschii_Gα             IFCAAISEYDQLLFEDETQNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
S.cereale_Gα              IFCAAISEYDQLLFEDETQNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   299
H.vulgare_Gα              IFCAAISEYDQLLFEDETQNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
B.distachyon_Gα           VFCAAISEYDQMLFEDEAQNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   299
O.sativa_Gα               IFCAAISEYDQMLFEDETKNRMMETKELFDWVLKQRCFEKTSFILFLNKFDIFEKKIQKV   300
S.italica Gα              IFCAAVSEYDQMLFEDETKNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
S.bicolor_Gα              IFCAAISEYDQMLCEDETKNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
Z.mays_Gα                 IFCAAISEYDQMLFEDETKNRMMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKV   300
A.thaliana_Gα             IFCAAISEYDQTLFEDEQKNRMMETKELFDWVLKQPCFEKTSFMLFLNKFDIFEKKVLDV   299
                                                                  G4     G5
T.aestivum_GA1-A          PLTVCEWFKDYEPIAPGK-QDVEHAYEFVKKKFEEVYFQSSKPERVDRVFKIYRTTALDQ   359
T.aestivum GA1-B          PLTVCEWFKDYEPIAPGK-QDVEHAYEFVKKKFEEVYFQSSKPDRVDRVFKIYGCSRSTE   359
T.aestivum_GA1-D          PLTVCEWFKDYEPIAPGK-QDVEHAYEFVKKKFEEVYFQSSKPERVDRVFKIYRTTALDQ   359
A.tauschii_Gα             PLTVCEWFKDYEPIAPGK-QDVEHAYEFVKKKFEEVYFQSSKPERVDRVFKIYRTTALDQ   359
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S.cereale_Gα           PLTVCEWFKDYEPIAPGK-QDVEHAYEFVKK--FQVYFQSSKPDLVDRVFKIYRTPREDQ   356
H.vulgare_Gα           PLTVCEWFKDYEPIAPGKVQDVEHAYEFVKKKFEEVYFQSSKPDRVDRVFKIYRTTALDQ   360
B.distachyon_Gα        PLTVCDWFKDYQPIAPGK-QDVEHAYEFVKKKFEELYFQSSKPDRVDRVFKIYRTTALDQ   358
O.sativa_Gα            PLSVCEWFKDYQPIAPGK-QEVEHAYEFVKKKFEELYFQSSKPDRVDRVFKIYRTTALDQ   359
S.italica Gα           PLSVCEWFKDYQPTAPGK-QEVEHAYEFVKKKFEELYFQSSKPDRVDRVFKIYRTTALDQ   359
S.bicolor_Gα           PLSACEWFKDYQPIAPGK-QEVEHAYEFVKKKFEELYFQSSKPDRVDRVFKIYRTTALDQ   359
Z.mays_Gα              PLSVCEWFKDYQPTAPGK-QEVEHAYEFVKKKFEELYFQSSKPDRVDRVFKIYRTTALDQ   359
A.thaliana_Gα          PLNVCEWFRDYQPVSSGK-QEIEHAYEFVKKKFEELYYQNTAPDRVDRVFKIYRTTALDQ   358

T.aestivum GA1-A       KLVKKTFKLMDESMRRSREGTGT--          382
T.aestivum_GA1-B       RRRWTRN-L----------------          367
T.aestivum_GA1-D       KLVKKTFKLMDESMRRSREGTGT--          382
A.tauschii_Gα          KLVKKTFKLMDESMRRSREGTGT--          382
S.cereale_Gα           KLVMKTFKLIDESMRGSREGTG---          378
H.vulgare_Gα           KLVKKTFKLIDESMRRSREGTGT--          383
B.distachyon Gα        KLVKKTFKLIDESMRRSREET----          379
O.sativa_Gα            KLVKKTFKLIDESMRRSREGT----          380
S.italica_Gα           KLVKKTFKLIDESMRRSREGT----          380
S.bicolor_Gα           KLVKKTFKLIDESMRRSREGT----          380
Z.mays_Gα              KLVKKTFKLIDESMRRSREGT----          380
A.thaliana_Gα          KLVKKTFKLVDETLRRRNLLEAGLL          383
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Supplementary Figure S2.1. Multiple sequence alignment of G protein α subunits from *Triticum aestivum* (A, B and D homeologs), monocot species *Aegilops tauschii, Hordeum vulgare, Secale cereale, Brachypodium distachyon, Setaria italica, Oryza sativa, Zea mays* ,*Sorghum bicolor* and dicot *Arabidopsis thaliana* by clustal omega. The conserved myristolated glycine (G) and S-acetylated cysteine (C) residues are represented by asterix (*) and conserved motifs G protein α subunits are represented as G1 to G5 are represented by continuous line (—) and two switches important for conformational change are represented by discontinuous lines (----).

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T.aestivum_Gβ-A      MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR   60
T.aestivum_Gβ-B      MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR   60
T.aestivum_Gβ-D      MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR   60
A.tauschi_Gβ         MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR   60
B.distachyon Gβ      MASVADLKEKHAAATASVNSLRERLRQRRQLLLDTDVERYSKAQGRTAVSFNQTDLVCCR   60
S.cereale_Gβ         MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR   60
H.vulgare_Gβ         MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR   60
O.sativa_Gβ          MASVAELKEKHAAATASVNSLRERLRQRRQMLLDTDVERYSRTQGRTPVSFNPTDLVCCR   60
S.italica_Gβ         MASVAELKEKHAAATASVNSLRERLRQRREMLLDTDVARYSKAQGRTPVSFNPTDLVCCR   60
S.bicolor Gβ         MASVAELKEKHAAATASVNSLRERLRQRRETLLDTDVARYSKSQGRLPVSFNPTDLVCCR   60
Z.mays_Gβ            MASVAELKEKHAAATASVNSLRERLRQRRETLLDTDVARYSKSQGRVPVSFNPTDLVCCR   60
A.thaliana_Gβ        -MSVSELKERHAVATETVNNLRDQLRQRRLQLLDTDVARYSAAQGRTRVSFGATDLVCCR   59
                                                    WD1
T.aestivum_Gβ-A      TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
T.aestivum_Gβ-B      TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
T.aestivum Gβ-D      TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
A.tauschi_Gβ         TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
B.distachyon_Gβ      TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
S.cereale_Gβ         TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
H.vulgare_Gβ         TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
O.sativa_Gβ          TPQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
S.italica Gβ         TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
S.bicolor_Gβ         TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNG   120
Z.mays_Gβ            TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNALTSQKTHAIKLHCPWVMACAFAPNG   120
A.thaliana_Gβ        TLQGHTGKVYSLDWTPERNRIVSASQDGRLIVWNALTSQKTHAIKLPCAWVMTCAFSPNG   119
                                WD2
T.aestivum_Gβ-A      QSVACGGLDSACSIFNLSSQADRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLITGSGDQ   180
T.aestivum Gβ-B      QSVACGGLDSACSIFNLSSQADRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLITGSGDQ   180
T.aestivum_Gβ-D      QSVACGGLDSACSIFNLSSQADRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLITGSGDQ   180
A.tauschi_Gβ         QSVACGGLDSACSIFNLSSQADRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLITGSGDQ   180
B.distachyon_Gβ      QSVACGGLDSACSIFNLNSQVDRDGNMPVSRILTGHKGYVSSCQYVPDQETRLITGSGDQ   180
S.cereale Gβ         QSVACGGLDSACSIFNLSTQADRDGNMPASRVLTGHKGYVSSCQYVPDQETRLITGSGDQ   180
H.vulgare Gβ         QSVACGGLDSACSIFNLSSQVDRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLITGSGDQ   180
O.sativa_Gβ          QSVACGGLDSACSIFNLNSQADRDGNIPVSRILTGHKGYVSSCQYVPDQETRLITSSGDQ   180
S.italica_Gβ         QSVACGGLDSACSIFNLNSQADRDGNMPVSRILTGHKGYVSSCQYVPDQESRLITSSGDQ   180
S.bicolor_Gβ         QSVACGGLDSACSIFNLNSQADRDGNMPVSRILTGHKGYVSSCQYVPDQETRLITSSGDQ   180
Z.mays Gβ            QSVACGGLDSACSIFNLNSQADRDGNMPVSRILTGHKGYVSSCQYVPDQETRLITSSGDQ   180
A.thaliana Gβ        QSVACGGLDSVCSIFSLSSTADKDGTVPVSRMLTGHRGYVSCCQYVPNEDAHLITSSGDQ   179
                WD3                                                     WD4
T.aestivum_Gβ-A      TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASR   240
T.aestivum_Gβ-B      TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASR   240
T.aestivum_Gβ-D      TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASR   240
A.tauschi_Gβ         TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASR   240
B.distachyon_Gβ      TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINPLNTNMFVSGSCDTTVRLWDLRIASR   240
S.cereale Gβ         TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASR   240
H.vulgare_Gβ         TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFVSGSCDTTVRLWDLRIASR   240
O.sativa_Gβ          TCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSSISNMFVSGSCDATVRLWDIRIASR   240
S.italica_Gβ         TCVLWDVTTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDATVRLWDIRIASR   240
S.bicolor_Gβ         TCVLWDVTTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDTTVRLWDIRIASR   240
Z.mays_Gβ            TCVLWDVTTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDTTVRLWDIRIASR   240
A.thaliana Gβ        TCILWDVTTGLKTSVFGGEFQSGHTADVLSVSISGSNPNWFISGSCDSTARLWDTRAASR   239

                                                    WD5
T.aestivum_Gβ-A      AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
T.aestivum_Gβ-B      AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
T.aestivum_Gβ-D      AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
A.tauschi_Gβ         AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
B.distachyon_Gβ      AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
S.cereale_Gβ         AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
H.vulgare_Gβ         AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPI   300
O.sativa_Gβ          AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDVRTGHQLQVYSREPDRNDNELPT   300
S.italica Gβ         AVRTYHGHEADVNSVKFFPDGHRFGTGSDDGTCRLFDMRTGHQLQVYSREPNRDDNELPT   300
S.bicolor_Gβ         AVRTYHGHEGDVNSVKFFPDGHRFGTGSDDGTCRLFDMRTGHQLQVYSRVPDRNDDELPT   300
Z.mays_Gβ            AVRTYHGHEDDVNSVKFFPDGHRFGTGSDDGTCRLFDMRTGHQLQVYSREPDRNSNELPT   300
A.thaliana_Gβ        AVRTFHGHEGDVNTVKFFPDGYRFGTGSDDGTCRLYDIRTGHQLQVYQPHGDG---ENGP   296
                                WD6
T.aestivum_Gβ-A      VTSVAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGTLQNSHEGRISCLGLSSDGSAL   359
T.aestivum Gβ-B      VTSVAFSISGRLLFAGYS-NGDCYVWDTLLAEMVLNLGTLQNSHEGRISCLGLSSDGSAL   359
T.aestivum_Gβ-D      VTSVAFSISGRLLFAGYS-NGDCYVWDTLLAEMVLNLGTLQNSHEGRISCLGLSSDGSAL   359
A.tauschi_Gβ         VTSVAFSISGRLLFAGYS-NGDCYVWDTLLAEMVLNLGTLQNSHEGRISCLGLSSDGSAL   359
B.distachyon_Gβ      VTSIAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGTLQNSHDGRISCLGLSSDGSAL   359
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S.cereale_Gβ          VTSVAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGTLQNSHEGRISCLGLSSDGSAL    359
H.vulgare_Gβ          VTSVAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGTLQNSHEGRISCLGLSSDGSAL    359
O.sativa_Gβ           VTSIAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGNLQNSHEGRISCLGLSSDGSAL    359
S.italica_Gβ          VTSIAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGNLQNSHDGRISCLGMSSDGSAL    359
S.bicolor_Gβ          VTSIAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGNLQNSHDGRISCLGMSSDGSAL    359
Z.mays_Gβ             VTSIAFSISGRLLFAGYS-NGDCYVWDTLLAEVVLNLGNLQNSHDGRISCLGMSSDGSAL    359
A.thaliana_Gβ         VTSIAFSVSGRLLFAGYASNNTCYVWDTLLGEVVLDLGLQQDSHRNRISCLGLSADGSAL    356

                                WD7
T.aestivum_Gβ-A       CTGSWDKNLKIWAFSGHRKIV     380
T.aestivum_Gβ-B       CTGSWDKNLKIWAFSGHRKIV     380
T.aestivum_Gβ-D       CTGSWDKNLKIWAFSGHRKIV     380
A.tauschi_Gβ          CTGSWDKNLKIWAFSGHRKIV     380
B.distachyon_Gβ       CTGSWDKNLKIWAFSGHRKIV     380
S.cereale_Gβ          CTGSWDKNLKIWAFSGHRKIV     380
H.vulgare_Gβ          CTGSWDKNLKIWAFSGHRKIV     380
O.sativa_Gβ           CTGSWDKNLKIWAFSGHRKIV     380
S.italica_Gβ          CTGSWDKNLKIWAFSGHRKIV     380
S.bicolor_Gβ          CTGSWDKNLKIWAFSGHRKIV     380
Z.mays_Gβ             CTGSWDKNLKIWAFSGHRKIV     380
A.thaliana_Gβ         CTGSWDSNLKIWAFGGHRRVI     377
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Supplementary Figure S2.2. Multiple sequence alignment of G protein β subunits from *Triticum aestivum* (A, B and D homeologs), monocot species *Aegilops tauschii, Hordeum vulgare, Secale cereale, Brachypodium distachyon, Setaria italica, Oryza sativa, Zea mays* ,*Sorghum bicolor* and dicot *Arabidopsis thaliana* by clustal omega. The conserved seven WD repeats are shown by continuous lines (—).

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O.sativa_Gγ2          MRGEANGEEEQQPPRRNHLRDDAEEEE----------------------------EVERR   32
S.bicolor_Gγ2         MRGQANGVEDRRPRGDDHEADDDEEDSEEEE-EEEGRHRGQGQGQGQGQGQGPPPQQRRHQQ   59
Z.mays_Gγ2            MRGQANGVEDRRQRGDDHEADNDGEEAEEEEGDDEGRHRG--------QGPP-Q-QRRHQ   50
S.italica_Gγ2         MRGEANGGEDRRPRGEDQEHEDDEEEE---------RRG------GEGAPPQRHVQAQRP   45
B.distachyon_Gγ2      MRGEANGEGRG-EEEQQ-----QQQ-------------------------VQ-EGEEADG   28
T.aestivum_Gγ2-A      MRGEANGGDRR-PRDEEEP-----P-------------------------QQ-PEEEQDR   28
H.vulgare_Gγ2         MRGEANGGDRR-LRDEDGE--EEEP-------------------------PQ-RQEEEER   31
T.aestivum_Gγ2-D      MRGEANGGDRR-PRDEEG--EEEEE-------------------------PP-QQQEEER   31
A.tauschiii_Gγ2       MRGEANGGDRR-PRDEEGEGEEEEE-------------------------PP-QQQEEER   33
T.aestivum_Gγ2-B      MRGEANGGDRR-PRDEEG--EEEEE-------------------------PP-QQQEEER   31
S.cereale_Gγ2         MRGEANGGDRR-PRDEEE--EEEEP-------------------------PP-QQQQEER   31
A.thaliana_Gγ1        ------------------------------------------------------------   0
A.thaliana_Gγ2        ------------------------------------------------------------   0
S.italica_Gγ1         ------------------------------------------------------------   0
S.bicolor_Gγ1         ------------------------------------------------------------   0
Z.mays_Gγ1            ------------------------------------------------------------   0
B.distachyon_Gγ1      ------------------------------------------------------------   0
O.sativa_Gγ1          ------------------------------------------------------------   0
H.vulgare_Gγ1         ------------------------------------------------------------   0
S.cereale_Gγ1         ------------------------------------------------------------   0
T.aestivum_Gγ1-D      ------------------------------------------------------------   0
T.aestivum_Gγ1-A      ------------------------------------------------------------   0
A.tauschiii_Gγ1       ------------------------------------------------------------   0
T.aestivum_Gγ1-B      ------------------------------------------------------------   0

O.sativa_Gγ2          AARPVSGQQQQQQRRRPTDVGGGAAMRSVGYVGKHRLSAAIARLDQELQSLQDELNELET   92
S.bicolor_Gγ2         TQRPSSGPQQQQQ--Q---HQPPPLTRNVGYVGKHRLSAAIARLDQELQSLQEELDELET   114
Z.mays_Gγ2            AQRPYSGPQQQ----P---RPPPPLARNVGYVGKHRLSAAIARFDQELQSLQDELDELET   103
S.italica_Gγ2         AARPSTDPQHQQH--P---PPPPGVMRNVGYVGKHRLSAAISRLDQELQSLQEELNELET   100
B.distachyon_Gγ2      AARPSSGQQQPAVAA-------AATTRGVGYVGKHRLSAAIARLDQELQSLQDELNELET   81
T.aestivum_Gγ2-A      TSRPSSGQQQQPAAAG---AA--ATTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELET   83
H.vulgare_Gγ2         AAKPSSGQQQQPAAAG---AATTTTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELET   88
T.aestivum_Gγ2-D      AARPSSEQQQP-VAAE---AAATTTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELET   87
A.tauschiii_Gγ2       AARPSSEQQQP-VAAE---AAATTTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELET   89
T.aestivum_Gγ2-B      AARPSSGQQQQQPAAA---GAATTTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELET   88
S.cereale_Gγ2         AARPSSGQEQQQPAAA---AAAATTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELET   88
A.thaliana_Gγ1        ------------MREETVV--YEQEESVSHGGGKHRILAELARVEQEVAFLEKELKEVEN   46
A.thaliana_Gγ2        ------------MEAGSSNSSGQLSGRVVDTRGKHRIQAELKRLEQEARFLEEELEQLEK   48
S.italica_Gγ1         ------------MQVGG---GGAGGGDAADIRGRHRIQAELKKLEQEARFLEEELEELQK   45
S.bicolor_Gγ1         ------------MQVGGG--GGGGGGDSADLRGRHRIQAELKKLEQEARFLEEELEELEK   46
Z.mays_Gγ1            ------------MQV------GDGGGDSADLRGRHRIQAELKKLEQEARFLEEELEELDK   42
B.distachyon_Gγ1      ------------MQVPGGGGGGGAGREAGDTRGRHRIQAELKKLEQEARFLKEELQELEK   48
O.sativa_Gγ1          -----------------MQAGG--GGDAGDTRGRHRIQAELKKLEQEARFLEEELEELDK   41
H.vulgare_Gγ1         ------------MQAPGG--VG--GGEAGDMRGRHRIQAELKKLEQEARFLEEELEKLNK   44
S.cereale_Gγ1         ------------MQVPGDVRAG--GGEAGDMRGRHRIQAELKKLEQETRFLEEELEELDK   46
```

Supplementary Figure S2.3. Multiple sequence alignment of G protein γ1 and γ2 subunits from *Triticum aestivum* (A, B and D homeologs), monocot species *Aegilops tauschii, Hordeum vulgare, Secale cereale, Brachypodium distachyon, Setaria italica, Oryza sativa, Zea mays ,Sorghum bicolor* and dicot *Arabidopsis thaliana* by clustal omega. The conserved, DPLL, -CaaX for Gγ1 motif (CXXL) and Gγ2 motifs (-KGSDFX) are shown.

```
A.thaliana_Gγ3        ------------------------------------------------------------   0
O.sativa_Gγ3          ---MAAAPRPKSPPAPPDPCGRHRLQLAVDALHREIGFLEGEINSIEGIHAASRCCREVD   57
S.italica_Gγ3         MAAAPAAPRPKSPPASPDPCGRHRLQLAVDALHREIGFLEGEISSIDGVHAASRCCKEVD   60
S.bicolor_Gγ3         ---MAAAPRPKSPPASPDPCGRHRLQLAVDALHREISFLEGEISSIEGVHAASRCCKEVD   57
Z.mays_Gγ3            -MAAAAAPRPKSPPASPDPCGRHRLQLAVDALHREIGFLEGEISSIEGVHAASRCCKEVD   59
O.sativa_Gγ4          ------------------------------------------------------------   0
B.distachyon_Gγ4      ------------------------------------------------------------   0
S.bicolor_Gγ4         ------------------------------------------------------------   0
Z.mays_Gγ4            ------------------------------------------------------------   0
Z.mays_Gγ5            ------------------------------------------------------------   0
S.italica_Gγ4         ------------------------------------------------------------   0
S.bicolor_Gγ5         ------------------------------------------------------------   0
B.distachyon_Gγ3      -MMAMVAPRPKSPPASPDPCGRHHLQLAVDTLHREIGFLEGEISSVEGVHAASKCCKEVD   59
T.aestivum_Gγ4-B      ------------------------------------------------------------   0
H.vulgare_Gγ4         ------------------------------------------------------------   0
S.cereale_Gγ3         ------------------------------------------------------------   0
T.aestivum-Gγ4-D      ------------------------------------------------------------   0
A.tauschiii_Gγ4       ------------------------------------------------------------   0
O.sativa_Gγ5          ------------------------------------------------------------   0
H.vulgare_Gγ3         ----MAAPRPKSP---LDPCGRHRLQLAVDALHRQISFLEGEINSIEGLHAASLCCKEVD   53
T.aestivum_Gγ3-B      ----MAAPRPKSP---LDPCGRRRLQLAVDALHRQISFLEGEISSIEGLHAASICCKEVD   53
T.aestivum_Gγ3-A      ----MAAPRPKSP---LDPCGRHRLQLAVDALHRQISFLEGEINSIEGLHAASICCKEVD   53
A.tauschiii_Gγ3       ----MAAPRPKSP---LDPCGRHRLQLAVDALHRQISFLEGEISSIEGLHAASICCKEVD   53
T.aestivum_Gγ3-D      ----MAAPRPKSP---LDPCGRHRLQLAVDALHRQISFLEGEISSIEGLHAASICCKEVD   53
T.aestivum_Gγ4-A      ------------------------------------------------------------   0


A.thaliana_Gγ3        ------------------------------------------------------------   0
O.sativa_Gγ3          EFIGRTPDPFITISSEKRSHDHSHHFLKKFRCLCRASACCLSYLSWICCCSSAAGGCSSS   117
S.italica_Gγ3         EFVGRNPDPFITIQPEKRSNEQSQQFLKKFRA----KSCLSY-LSWICCGGGG-------   108
S.bicolor_Gγ3         EFVGSNPDPFLTIQPEKGSHDQSQQFLKKFRA----KSCLSYYLSWICCCGGGGGGGSGG   113
Z.mays_Gγ3            EFVGRNPDPFLTIQQERGSHDQSQQFLKKFRG----KSCLSYYLSWIC--------GGGW   107
O.sativa_Gγ4          ------------------------------------------------------------   0
B.distachyon_Gγ4      ------------------------------------------------------------   0
S.bicolor_Gγ4         ------------------------------------------------------------   0
Z.mays_Gγ4            ------------------------------------------------------------   0
Z.mays_Gγ5            ------------------------------------------------------------   0
S.italica_Gγ4         ------------------------------------------------------------   0
S.bicolor_Gγ5         ------------------------------------------------------------   0
B.distachyon_Gγ3      EFVGKNADPFITISSKKANTDQSRHLPKKFRA----R-TCLSYLSWMCCCGGC-------   107
T.aestivum_Gγ4-B      ------------------------------------------------------------   0
H.vulgare_Gγ4         ------------------------------------------------------------   0
S.cereale_Gγ3         ------------------------------------------------------------   0
T.aestivum_Gγ4-D      ------------------------------------------------------------   0
A.tauschiii_Gγ4       ------------------------------------------------------------   0
O.sativa_Gγ5          ------------------------------------------------------------   0
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H.vulgare_Gγ3          EFIGKNADPLITIPSEKGNTNQSHRSAKKIRA----RWACLSCFPWMC-GGWC-------   101
T.aestivum_Gγ3-B       EFIGKNADPFITISSEKGNADQSHRSPKKIRT----RWACLSCFPWIC-GGGC-------   101
T.aestivum_Gγ3-A       EFIGKNADPFITISSEKGNAEQSHPFPKKIRT----RWACLSCFPWIC-GGGC-------   101
A.tauschiii_Gγ3        EFIGKNADPFITISSEKGNADQSHRFPKKIRT----RWACLSCFPWIC-GGGC-------   101
T.aestivum_Gγ3-D       EFIGKNADPFITISSEKGNADQSHRFPKKIRT----RWACLSCFPWIC-GGGC-------   101
T.aestivum-Gγ4-A       ------------------------------------------------------------   0

A.thaliana_Gγ3         ------------------------------------------------------------   0
O.sativa_Gγ3           S-SSSFNLKRPSCCCNCNCNCCSSSSSSCGAALTKSPCRCRRRSCCCRRCC-CGGVGVRA   175
S.italica_Gγ3          -CP-PFQLKTTMRPP--------SASCSCGGARLRKLCS---SPCCCCCCCRCRVVYA--   153
S.bicolor_Gγ3          WCPPSLQL---KRPA--------APSCSCA-PRLRKLC-------CCCCCCRCRVVYAGG   154
Z.mays_Gγ3             WCPPPLQL---KRPP--------APSCSCA-PRLGKLCSSTASSCCSCCCCRFRVVYA--   153
O.sativa_Gγ4           ------------------------------------------------------------   0
B.distachyon_Gγ4       ------------------------------------------------------------   0
S.bicolor_Gγ4          ------------------------------------------------------------   0
Z.mays_Gγ4             ------------------------------------------------------------   0
Z.mays_Gγ5             ------------------------------------------------------------   0
S.italica_Gγ4          ------------------------------------------------------------   0
S.bicolor_Gγ5          ------------------------------------------------------------   0
B.distachyon_Gγ3       ---PSVQLQGPTSCCSC--------------GALGGLCGCCSTGECCRCR-----VGCGG   145
T.aestivum_Gγ4-B       ------------------------------------------------------------   0
H.vulgare_Gγ4          ------------------------------------------------------------   0
S.cereale_Gγ3          ------------------------------------------------------------   0
T.aestivum-Gγ4-D       ------------------------------------------------------------   0
A.tauschiii_Gγ4        ------------------------------------------------------------   0
O.sativa_Gγ5           ------------------------------------------------------------   0
H.vulgare_Gγ3          ---SAVQRKGPSCCCGC--------------PRC---------------C-----VGSGG   124
T.aestivum_Gγ3-B       ---SAVQLKGPSCCCGC--------------PRC---------------C-----AGSGG   124
T.aestivum_Gγ3-A       ---SAVQLKGPSCCCGC--------------PRC---------------C-----AGSGG   124
A.tauschiii_Gγ3        ---SAVQLKGLSCCCGC--------------PRC---------------C-----VGSGG   124
T.aestivum_Gγ3-D       ---SAVQLKGPSCCCGC--------------PRC---------------C-----VGSGG   124
T.aestivum-Gγ4-A       ------------------------------------------------------------   0

A.thaliana_Gγ3         ----------------------------------------MSAPSGGGE---GGGKESAA   17
O.sativa_Gγ3           CASCSC---SPPCACCAPPCAGCSCRCTCPCPCPGGCSCACPAC-R------CCCGVPRC   225
S.italica_Gγ3          ----G---------C-CAPCPRCSCG--CA--CPRCSSCACCPTCS------DACCAPRC   189
S.bicolor_Gγ3          AGGCG---------C-CAPCPRCSCDCTCA--CPRCCSC--PRCSA------ACCCAPRC   194
Z.mays_Gγ3             AAGCG---------C-CAPCPRCSCDCTCA--CPRCCSCACPMCSA-------ACCAPRC   194
O.sativa_Gγ4           ------------------------------------------------------------   0
B.distachyon_Gγ4       ------------------------------------------------------------   0
S.bicolor_Gγ4          ------------------------------------------------------------   0
Z.mays_Gγ4             ------------------------------------------------------------   0
Z.mays_Gγ5             ------------------------------------------------------------   0
S.italica_Gγ4          ------------------------------------------------------------   0
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```
S.bicolor_Gγ5          ------------------------------------------------------------   0
B.distachyon_Gγ3       CGCCCCCCRGSPCR-SRTPSPRCSCGCTCSCPSCCSSSCACPAPSC--------CRAPRC   196
T.aestivum_Gγ4-B       ------------------------------------------------------------   0
H.vulgare_Gγ4          ------------------------------------------------------------   0
S.cereale_Gγ3          ------------------------------------------------------------   0
T.aestivum-Gγ4-D       ------------------------------------------------------------   0
A.tauschiii_Gγ4        ------------------------------------------------------------   0
O.sativa_Gγ5           ------------------------------------------------------------   0
H.vulgare_Gγ3          CGG------------------GPSCGCTCSCAGC-SSSCSCPACASCGAACCGCVPRPRC   165
T.aestivum_Gγ3-B       CGGG-----------------GPSCGCSCSCAGC-SSSCACPACAGCGPACCGGVPRPRC   166
T.aestivum_Gγ3-A       CGG------------------GPSCGCSCSCAGC-SSSCACPACAGCGTVCCGGVPRPRC   165
A.tauschiii_Gγ3        CGG------------------GPSCGCSCSCAGC-SSSCACPACAGCGAACCGGAPRPRC   165
T.aestivum_Gγ3-D       CGG------------------GPSCGCSCSCAGC-SSSCACPACAGCGAACCGGAPRPRC   165
T.aestivum_Gγ4-A       ------------------------------------------------------------   0


A.thaliana_Gγ3         GGVSSSSLAPSSLPPPRPKSPPEYPDLYGKRREAARVQMLEREIGFLEGEIKFIEGVQPA   77
O.sativa_Gγ3           CPPCL--------------------------------------------------------   230
S.italica_Gγ3          CLCL---------------------------------------------------------   193
S.bicolor_Gγ3          CLCL---------------------------------------------------------   198
Z.mays_Gγ3             CLCL---------------------------------------------------------   198
O.sativa_Gγ4           -----------MGEAPRPKSPPRYPDLCGRRRLQLEMQILNREVGFLEQELQGLERIQPV   49
B.distachyon_Gγ4       -----------MGEAPRPKSPPKYPDLCGRRRLQLEVQSLNREVGFLEQELQGLERMQPV   49
S.bicolor_Gγ4          -----------MGEAPQPKSPPRYPDLCGRRRLQLEVQILNREVGFLEQEIRGLERIQPV   49
Z.mays_Gγ4             -----------MGEAPQPKSPPRYPDLCGRRRLQLEVQILNREVGFLEQEIQGLERIQPV   49
Z.mays_Gγ5             ----MGEEVAVVLEPPRPKSPPRYPDLCGRRRLQLELQALNREIDFLKDELQSLEGVPPV   56
S.italica_Gγ4          ----MGEQVAVVLEPPRPKSPPRYPDLCGRRRLQLELQILNREVDFLKDELQSLEGVPPV   56
S.bicolor_Gγ5          MGEEVAAAAAVVLEPPRPKSPPRYPDLCGRRRLQLELQILNREIDFLKDELQSLEGVPPV   60
B.distachyon_Gγ3       CYLCS--------------------------------------------------------   201
T.aestivum_Gγ4-B       ----MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   56
H.vulgare_Gγ4          ----MGEGAVVVLEPPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   56
S.cereale_Gγ3          ----MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   56
T.aestivum-Gγ4-D       ----MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   56
A.tauschiii_Gγ4        ----MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   56
O.sativa_Gγ5           ----MGEE-AVVMEAPRPKSPPRYPDLCGRRRMQLEVQILSREITFLKDELHFLEGAQPV   55
H.vulgare_Gγ3          CLCS---------------------------------------------------------   169
T.aestivum_Gγ3-B       CLCS---------------------------------------------------------   170
T.aestivum_Gγ3-A       CLCS---------------------------------------------------------   169
A.tauschiii_Gγ3        CLCS---------------------------------------------------------   169
T.aestivum_Gγ3-D       CLCSMGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   225
T.aestivum_Gγ4-A       ----MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPV   56


A.thaliana_Gγ3         SRC--IKEVSDFVVANSDPLIPAQRKSRRSFRFWKWLCGPCLSLVSFCCCCQSKCSCHLR   135
O.sativa_Gγ3           --------------------------------------------------------------   230
S.italica_Gγ3          --------------------------------------------------------------   193
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```
S.bicolor_Gγ3          ------------------------------------------------------------  198
Z.mays_Gγ3             ------------------------------------------------------------  198
O.sativa_Gγ4           SRC--CKDVNEFVGAKSDPLIPINKRKHRSCSLYRWIRSKLCNCLLC-LCCWCRCLPKPK  106
B.distachyon_Gγ4       SRC--CKDVNEYVGAKTDPLIPINKRKHRSCSLYRWIRSKLCTCFSC-LCCWCRCLPK--  104
S.bicolor_Gγ4          SRC--CKDVNEFVSAKTDPMIPVSKRKHGSCSFSRWIRSKLRTCFSC-LCC---------  97
Z.mays_Gγ4             SRC--CNDVNEFVSAKTDPMIPVSKRRHGSCSFSRWIRSKLRTCFSC-LCCWCHCLPKPN  106
Z.mays_Gγ5             SRS--CKEVNEFVGTKQDPLIPIKKKTHRSCRLFWWIRSKLCICVSW-FCCSCHCLPSCK  113
S.italica_Gγ4          SRS--CKEVNEFVGTKQDPLLPIEKKRHRSCGLFWWIGSKLCICVPW-ICCSCQCLPKCK  113
S.bicolor_Gγ5          SRS--CKEVNEFVGTKQDPLLPIKKKTHRSCRLFWWIRSKLCICVSW-FCCSCHCLPNCK  117
B.distachyon_Gγ3       ------------------------------------------------------------  201
T.aestivum_Gγ4-B       SRSGCLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASW-LCCSCQCLPTCK  115
H.vulgare_Gγ4          SRSACLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCVCASW-LCCSCQCLPTCK  115
S.cereale_Gγ3          SRSGCLKEVNEFVGTKQDPLIPINKTKHRSCRLYWWIRSKLCICASW-LCCSCQCLPTCK  115
T.aestivum-Gγ4-D       SRSGCLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASW-LCCSCQCLPTCK  115
A.tauschiii_Gγ4        SRSGCLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASW-LCCSCQCLPTCK  115
O.sativa_Gγ5           SRSGCIKEINEFVGTKHDPLIPTKRRRHRSCRLFRWIGSKLCICISC-LCYCCKCSPKCK  114
H.vulgare_Gγ3          ------------------------------------------------------------  169
T.aestivum_Gγ3-B       ------------------------------------------------------------  170
T.aestivum_Gγ3-A       ------------------------------------------------------------  169
A.tauschiii_Gγ3        ------------------------------------------------------------  169
T.aestivum_Gγ3-D       SRSGCLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASW-LCCSCQCLPTCK  284
T.aestivum-Gγ4-A       SRSGCLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASW-LCCSCQCLPTCK  115

A.thaliana_Gγ3         KPKCCNCTSCSCIGSKCCDGSCCSN---------------ICCCPRLSCPSCSCFRGCWC  180
O.sativa_Gγ3           ------------------------------------------------------------  230
S.italica_Gγ3          ------------------------------------------------------------  193
S.bicolor_Gγ3          ------------------------------------------------------------  198
Z.mays_Gγ3             ------------------------------------------------------------  198
O.sativa_Gγ4           KPSCFSCSCCSCCDTSCCRPSCGCLKAPSS-----------CCCKS--NCS---------  144
B.distachyon_Gγ4       RPSCFTCSCCSCGDTSCCTPSCSCLNKTPS-----------CCKP-----Q---------  139
S.bicolor_Gγ4          ----FSCSCCTCRATQCCTPPTCSCPKTPS-----------CSSC-----C---------  128
Z.mays_Gγ4             APSCFSCSCCTCRDTQCCTP-ICRCSKTPS-----------C-------------------  136
Z.mays_Gγ5             RPCCLDCSCCSCPDLSCCKPSCKSCNKPCFGPNSCSCCDISCCKPD--CPSCS---SNC-  167
S.italica_Gγ4          RPCCFDCSCCSCPDVSCCKPSCKSCNKPCCGPNSCSCCNVSCCKPD--CPSCS---PSCS  168
S.bicolor_Gγ5          RPCCLDCSCCSCPDLSCCKPSCKSCNKPCSWPNSCSCCDTPCCKPD--CPSCS---SSCS  172
B.distachyon_Gγ3       ------------------------------------------------------------  201
T.aestivum_Gγ4-B       RPRCFDCSCC--------EPNCS------------------CCSLN--C-----------  136
H.vulgare_Gγ4          RPSCLDCSCC--------EPNCS------------------CCSPN--C-----------  136
S.cereale_Gγ3          RPRCFDCSCC--------EPNCS------------------CCSPN--C-----------  136
T.aestivum-Gγ4-D       RPMCLDCSCC--------KPNCS------------------CCSPN--C-----------  136
A.tauschiii_Gγ4        RPMCLDCSCC--------KPNCS------------------CCSPN--C-----------  136
O.sativa_Gγ5           RPRCLNCSCSSCCDEPCCKPNCSAC------------CAGSCCSPD--CCSC--------  152
H.vulgare_Gγ3          ------------------------------------------------------------  169
T.aestivum_Gγ3-B       ------------------------------------------------------------  170
T.aestivum_Gγ3-A       ------------------------------------------------------------  169
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A.tauschiii_Gγ3       ------------------------------------------------------------   169
T.aestivum_Gγ3-D      RPRCFDCSCC--------EPNCS------------------CCSPN--CCS---------   307
T.aestivum-Gγ4-A      RPRCFDCSCC--------EPNCS------------------CCSPN--CCS---------   138

A.thaliana_Gγ3        SCPDMSCCIPSCFRSCSC------------TRPSCLNKKKSSCCSCNC-----KIRWSSC   223
O.sativa_Gγ3          ------------------------------------------------------------   230
S.italica_Gγ3         ------------------------------------------------------------   193
S.bicolor_Gγ3         ------------------------------------------------------------   198
Z.mays_Gγ3            ------------------------------------------------------------   198
O.sativa_Gγ4          ------------------------------------------------------------   144
B.distachyon_Gγ4      ------------------------------------------------------------   139
S.bicolor_Gγ4         ------------------------------------------------------------   128
Z.mays_Gγ4            ------------------------------------------------------------   136
Z.mays_Gγ5            ------------SSCCTGSPSCCKPNCNTCCRPNCSSCCDPSCCKPN-------------   202
S.italica_Gγ4         SCC-----KPNCSSC--CSPSCCKPNCSTCC-------------KPNCG-----SCCKPK   203
S.bicolor_Gγ5         SCCNLSCCNPNCSSCCTCNPSCCKPNCNSCCRPNCSSCCNPSCCKPNCG-----SWWKPS   227
B.distachyon_Gγ3      ------------------------------------------------------------   201
T.aestivum_Gγ4-B      ------------CSC--FSIPSCCKP----------------------SCGCFECC------   156
H.vulgare_Gγ4         ------------CSC--FKIPSCCKP----------------------SCGCFGCC------   156
S.cereale_Gγ3         ------------CSC--FKIPSCCKP----------------------SCGCFDCC------   156
T.aestivum-Gγ4-D      ------------CSC--FKIPSCCKP----------------------SCGCFECC------   156
A.tauschiii_Gγ4       ------------CSC--FKIPSCCKP----------------------SCGCFECC------   156
O.sativa_Gγ5          -------CKPNCSC--CKTPSCCKPNCSC-SCPSCSSCCDTSCCKPSCTCFNIFSCFKSL   202
H.vulgare_Gγ3         ------------------------------------------------------------   169
T.aestivum_Gγ3-B      ------------------------------------------------------------   170
T.aestivum_Gγ3-A      ------------------------------------------------------------   169
A.tauschiii_Gγ3       ------------------------------------------------------------   169
T.aestivum_Gγ3-D      ------------CSC--FKIPPCCKP----------------------SCGCFDCCS-----   328
T.aestivum-Gγ4-A      ------------CSC--FKIPPCCKP----------------------SCGCFDCCS-----   159

A.thaliana_Gγ3        FSCPKVRLCSCCFCNCKN----LCSNPCCLAF-----------------------------   251
O.sativa_Gγ3          ------------------------------------------------------------   230
S.italica_Gγ3         ------------------------------------------------------------   193
S.bicolor_Gγ3         ------------------------------------------------------------   198
Z.mays_Gγ3            ------------------------------------------------------------   198
O.sativa_Gγ4          ---------------CCSSDCCTCSLPSCG-----CTGCG--HCRPL-CGGGG-------   174
B.distachyon_Gγ4      ---------------CGGGSSDCCSLPSCCDCKTHCTGCG--DCHCQ-PQ----------   171
S.bicolor_Gγ4         ---------------CSPGWC-SCSLPSCS-C----TGCG--HCRTQ-CG----------   154
Z.mays_Gγ4            ----------------SPGCC-TCSLPSCS-CK-TPPGCG--HCRPQ-CS----------   164
Z.mays_Gγ5            CSCFKTLSCCKFQC---SPNCCTCSLPSCSG---------CNPCG---------------   235
S.italica_Gγ4         CSCFGLPSCCKFQC---SPNCCTCTMPSCSG---------CNPCG---------------   236
S.bicolor_Gγ5         CSCFKAPSCCKLQC---SPNCCTCSLPRCSG---------CNPCG---------------   260
B.distachyon_Gγ3      ------------------------------------------------------------   201
T.aestivum_Gγ4-B      ----------------------SCSKPQCCSSGC--------------------------   168
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H.vulgare_Gγ4          ----------------------SCSKPQCCSGGC-------------------------   168
S.cereale_Gγ3          ----------------------SCSKPQCCSSGC-------------------------   168
T.aestivum-Gγ4-D       ----------------------SCSKPQCCSSGC-------------------------   168
A.tauschiii_Gγ4        ----------------------SCSKPQCCSSGC-------------------------   168
O.sativa_Gγ5           YSCFKIPSCFKSQCNCSSPNCCTCTLPSCSCKGCACPSCGCNGCGCPSCGCNGCGCPSCG   262
H.vulgare_Gγ3          ------------------------------------------------------------   169
T.aestivum_Gγ3-B       ------------------------------------------------------------   170
T.aestivum_Gγ3-A       ------------------------------------------------------------   169
A.tauschiii_Gγ3        ------------------------------------------------------------   169
T.aestivum_Gγ3-D       ----------------------CSCSKPQCCS-------GGC--------------------   341
T.aestivum-Gγ4-A       ----------------------CSCSKPQCCS-------GGC--------------------   172


A.thaliana_Gγ3         ------------------------------------------------------------   251
O.sativa_Gγ3           ------------------------------------------------------------   230
S.italica_Gγ3          ------------------------------------------------------------   193
S.bicolor_Gγ3          ------------------------------------------------------------   198
Z.mays_Gγ3             ------------------------------------------------------------   198
O.sativa_Gγ4           --GCCPPSD--------CCSSCKCSCSSCTRCCSSCAGGCKPSCSGC-GTGCSSCGG---   220
B.distachyon_Gγ4       --CCCKPSC--------SCSLPSCCCSLSS----------------------GSSC-----   195
S.bicolor_Gγ4          --NCCPSGC--------SCADCPCSCSCPS----------------------CVSC-----   178
Z.mays_Gγ4             --SCCSSGC--------SCADCPCSCSCPP----------------------CCS------   187
Z.mays_Gγ5             ----------------------SCKK----CWS-CPSDC---KPSC-GCFSAQCCSCAGCC-   265
S.italica_Gγ4          ----------------------SCKG----CCS-CPSDCCNRKPNC-SCFSAQCCSCAECYS   270
S.bicolor_Gγ5          ----------------------SCKQ----CCS-CPTDCCNCKPSC-GCFSAQCCSCAAC--   292
B.distachyon_Gγ3       ------------------------------------------------------------   201
T.aestivum_Gγ4-B       ----------------NPCGECKPECGSCSG-GGC-C-----------GEQ-----C---   191
H.vulgare_Gγ4          ----------------NPCGECKPECGSCSA--GGCCGDCKPSC-SCCGEQCQ---C---   203
S.cereale_Gγ3          ----------------NPCGECKPECGSCSG--GGCCGDCKPSC-SCCGEQCCSCGG---   206
T.aestivum-Gγ4-D       ----------------NPCGECKPECGSCSG-GGGCCGDCKPSC-SCCGEQCCSCAG---   207
A.tauschiii_Gγ4        ----------------NPCGECKPECGSCSG-GGGCCGGCKPSC-SCCGEQCCSCAG---   207
O.sativa_Gγ5           CNGCGLPSCGCNGCGSCSCAQCKPDCGSCST-N-C--CSCKPSCNGCCGEQCCRCADCFS   318
H.vulgare_Gγ3          ------------------------------------------------------------   169
T.aestivum_Gγ3-B       ------------------------------------------------------------   170
T.aestivum_Gγ3-A       ------------------------------------------------------------   169
A.tauschiii_Gγ3        ------------------------------------------------------------   169
T.aestivum_Gγ3-D       ----------------NLCGECKPECGSCSG-G-GCCGDCKPSC-SCCGEQCCSCAG---   379
T.aestivum-Gγ4-A       ----------------NLCGECKPECGSCSG-G-GCCGDCKPSC-SCCGEQCCSCAG---   210


A.thaliana_Gγ3         ------------------------------------------------------------   251
O.sativa_Gγ3           ------------------------------------------------------------   230
S.italica_Gγ3          ------------------------------------------------------------   193
S.bicolor_Gγ3          ------------------------------------------------------------   198
Z.mays_Gγ3             ------------------------------------------------------------   198
O.sativa__Gγ4          ---GCCPKCSSCAAP-------CVGCLA------LLRRWLSCRSSCCKG-QPSCCKCQSS   263
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B.distachyon_Gγ4      ----GCAEKCSCTPC--------LGCLG-----VFFERCLSCRSSCCKGQQPSCCKCQLS   238
S.bicolor_Gγ4         -----CPGCFSCAGC-------SAGCLG------ALNRCLSCVSSCCSGMRPSCCKCQSS   220
Z.mays_Gγ4            -----CPGFFSCEGC-------SAGCLG------ALNRCLGGLSSCCSEMRPSCCKCQSS   229
Z.mays_Gγ5            ----SCSSCFSCFGCFKSFKCSN-----LFG-CCSCKQCFKCQSSCCKG-ASSCCKCQSS   314
S.italica_Gγ4         CTCPSCSSCFSCFGCFKSWKCSN-----LFGGCCSCKQCFKCQSSCCKG-APSCCKCQSS   324
S.bicolor_Gγ5         --CSSCLSCFSCFGCFKSFKCSN-----LFG-CCSCKQCFKCQSSCCKG-APSCCKCQSS   343
B.distachyon_Gγ3      ------------------------------------------------------------   201
T.aestivum_Gγ4-B      CSCPRCTG---CFSCFKVPKCSC-------------AQCFNCQSSCCKG-QPSCFRCQSS   234
H.vulgare_Gγ4         CSCPRCTG-----GCFKLPKCSC-------------AQCFNCQSSCCKG-QPSCFRCQSS   244
S.cereale_Gγ3         CSCPRCAG-----GCFKLPKCSC-------------AQCFNCQSSCCKG-QPSCFRCQSS   247
T.aestivum-Gγ4-D      CSCPRCTG-----GCFKLPKCSC-------------ARCFNCQSSCCKG-QPSCFRCQSS   248
A.tauschiii_Gγ4       CSCPRCTG-----GCFKLPKCSC-------------ARCFNCQSSCCKG-QPSCFRCQSS   248
O.sativa_Gγ5          CSCPRC------SSCFNIFKCSCAGCCSSLCKCPCTTQCFSCQSSCCKR-QPSCCKCQSS   371
H.vulgare_Gγ3         ------------------------------------------------------------   169
T.aestivum_Gγ3-B      ------------------------------------------------------------   170
T.aestivum_Gγ3-A      ------------------------------------------------------------   169
A.tauschiii_Gγ3       ------------------------------------------------------------   169
T.aestivum_Gγ3-D      CSCPRCTGG--CLSCFKLPKCSC-------------ARCFNCQSSCCKG-QPSCFRCQSS   423
T.aestivum-Gγ4-A      CSCPRCTGG--CLSCFKLPKCSC-------------ARCFNCQSSCCKG-QPSCFRCQSS   254


A.thaliana_Gγ3        ------------------------------------------------------------   251
O.sativa_Gγ3          ------------------------------------------------------------   230
S.italica_Gγ3         ------------------------------------------------------------   193
S.bicolor_Gγ3         ------------------------------------------------------------   198
Z.mays_Gγ3            ------------------------------------------------------------   198
O.sativa_Gγ4          CCEGEPSCCCCCGGGKGSSACCCGRPCCLGGATPAPSCPECSCGCSCSCPRCKDGC-SRP   322
B.distachyon_Gγ4      CCEGDESSSCC-GRGACD----SC-KSCFG----APSCPECSCGCVCSCPRCKGGC-RCP   287
S.bicolor_Gγ4         CCEAGSSSSSCRGTGTGA----CCRGSCLGAPAATPSCPECSCGCVCSCSRCKGGCCHCP   276
Z.mays_Gγ4            CCEGGS---SCRGTGTGA----CCRGSCLGAPA--SSCPECSCGCVCSCSRCKGGC-RCP   279
Z.mays_Gγ5            CCEGEDGSSCCRR-------------PCC--TFPKPACSGCSCGCAWSCRKCTEWC-RCS   358
S.italica_Gγ4         CCEGEDGSSCCWR-------------SCC--SVPKPSCPGCSCGCVWSCKKCTEGC-RCS   368
S.bicolor_Gγ5         CCEGEDGSSCCRR-------------SCC--SVPKPACPGCSCGCVWSCKKCTDGC-RCS   387
B.distachyon_Gγ3      ------------------------------------------------------------   201
T.aestivum_Gγ4-B      CCDKG--G-----------------------------------------------------   240
H.vulgare_Gγ4         CCDKG--GCCSSG-------------SCL--SCPKPSCPECSCGCVWSCKNCTDGC-RCA   286
S.cereale_Gγ3         CCDKG--GCCSGG-------------SCV--SCPKPSCPECSCGCVWSCKNCTDGC-RCA   289
T.aestivum-Gγ4-D      CCDKG--GCCSGG-------------SCL--SCPKPSCPECSCGCVWSCKNCTDGC-RCA   290
A.tauschiii_Gγ4       CCDKG--GCCSGG-------------SCL--SCPKPSCPECSCGCVWSCKNCTDGC-RCA   290
O.sativa_Gγ5          CCEGQ--PSCCEG-------------HCC--SLPKPSCPECSCGCVWSCKNCTEGC-RCP   413
H.vulgare_Gγ3         ------------------------------------------------------------   169
T.aestivum_Gγ3-B      ------------------------------------------------------------   170
T.aestivum_Gγ3-A      ------------------------------------------------------------   169
A.tauschiii_Gγ3       ------------------------------------------------------------   169
T.aestivum_Gγ3-D      CCDKG--GCCSGG-------------SCL--SCPKPSCPECSCGCVWSCKNCTDGC-RCA   465
T.aestivum-Gγ4-A      CCDKG--GCCSGG-------------SCL--SCPKPSCPECSCGCVWSCKNCTDGC-RCA   296
```

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A.thaliana_Gγ3          --------------      251
O.sativa_Gγ3           --------------       230
S.italica_Gγ3           --------------      193
S.bicolor_Gγ3           --------------      198
Z.mays_Gγ3              --------------      198
O.sativa_Gγ4            SCG-NPCCAGGCLC      335
B.distachyon_Gγ4        SCG-NPCGAGGCLC      300
S.bicolor_Gγ4           SCGNNPCCAGGCLC      290
Z.mays_Gγ4              SCGSNPCCPGGCLC      293
Z.mays_Gγ5              GCR-NPCCATGCLC      371
S.italica_Gγ4           GCR-NPCCATGCLC      381
S.bicolor_Gγ5           GCR-NPCCATGCLC      400
B.distachyon_Gγ3        --------------      201
T.aestivum_Gγ4-B        --------------      240
H.vulgare_Gγ4           RCC-A----SGCLC      295
S.cereale_Gγ3           RCC-A----GGCLC      298
T.aestivum-Gγ4-D        RCC-A----GGCLC      299
A.tauschiii_Gγ4         RCC-A----GGCLC      299
O.sativa_Gγ5            RCR-NPCCLSGCLC      426
H.vulgare_Gγ3           --------------      169
T.aestivum_Gγ3-B        --------------      170
T.aestivum_Gγ3-A        --------------      169
A.tauschiii_Gγ3         --------------      169
T.aestivum_Gγ3-D        RCC-A----GGCLC      474
T.aestivum-Gγ4-A        RCC-A----GGCLC      305
```
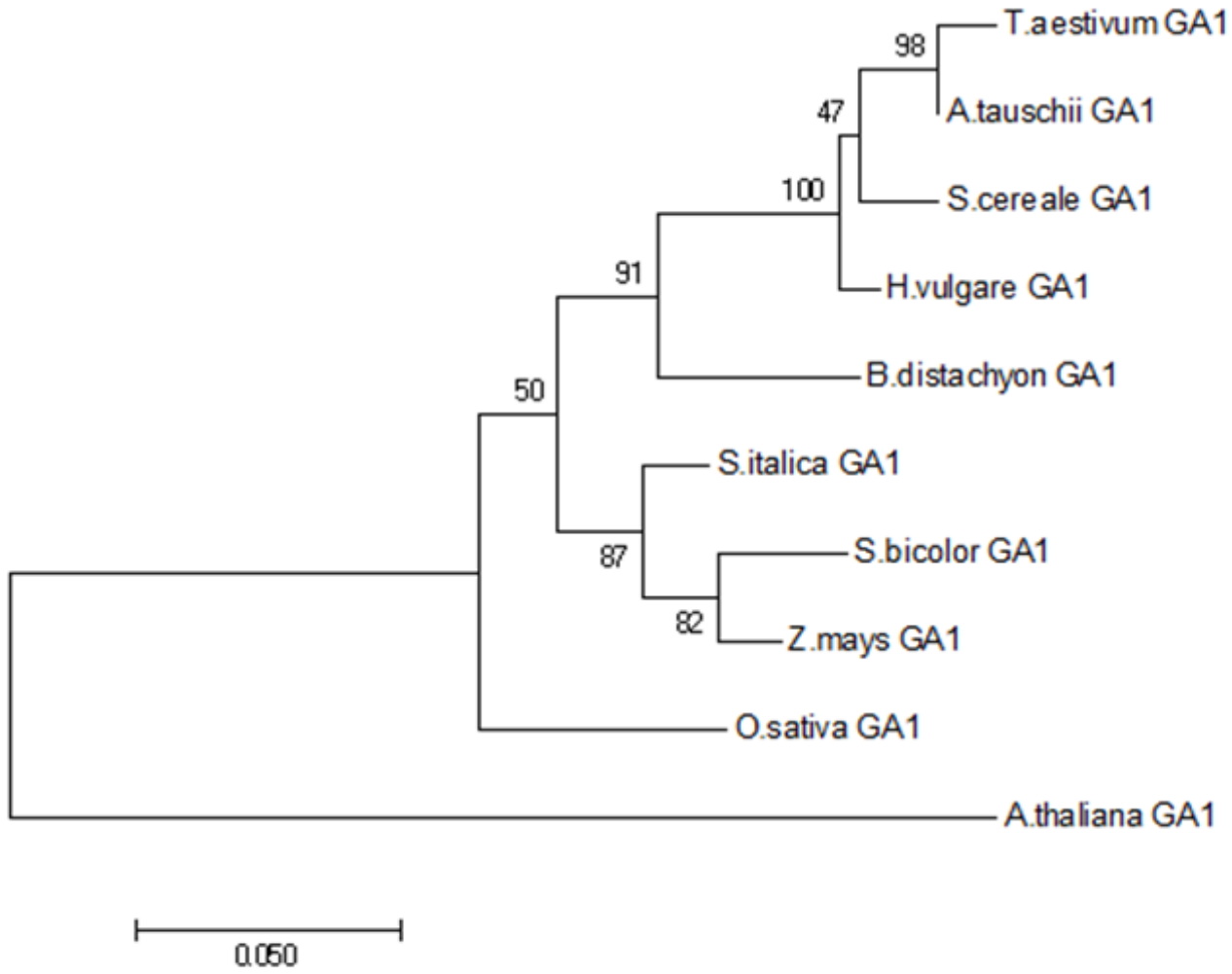
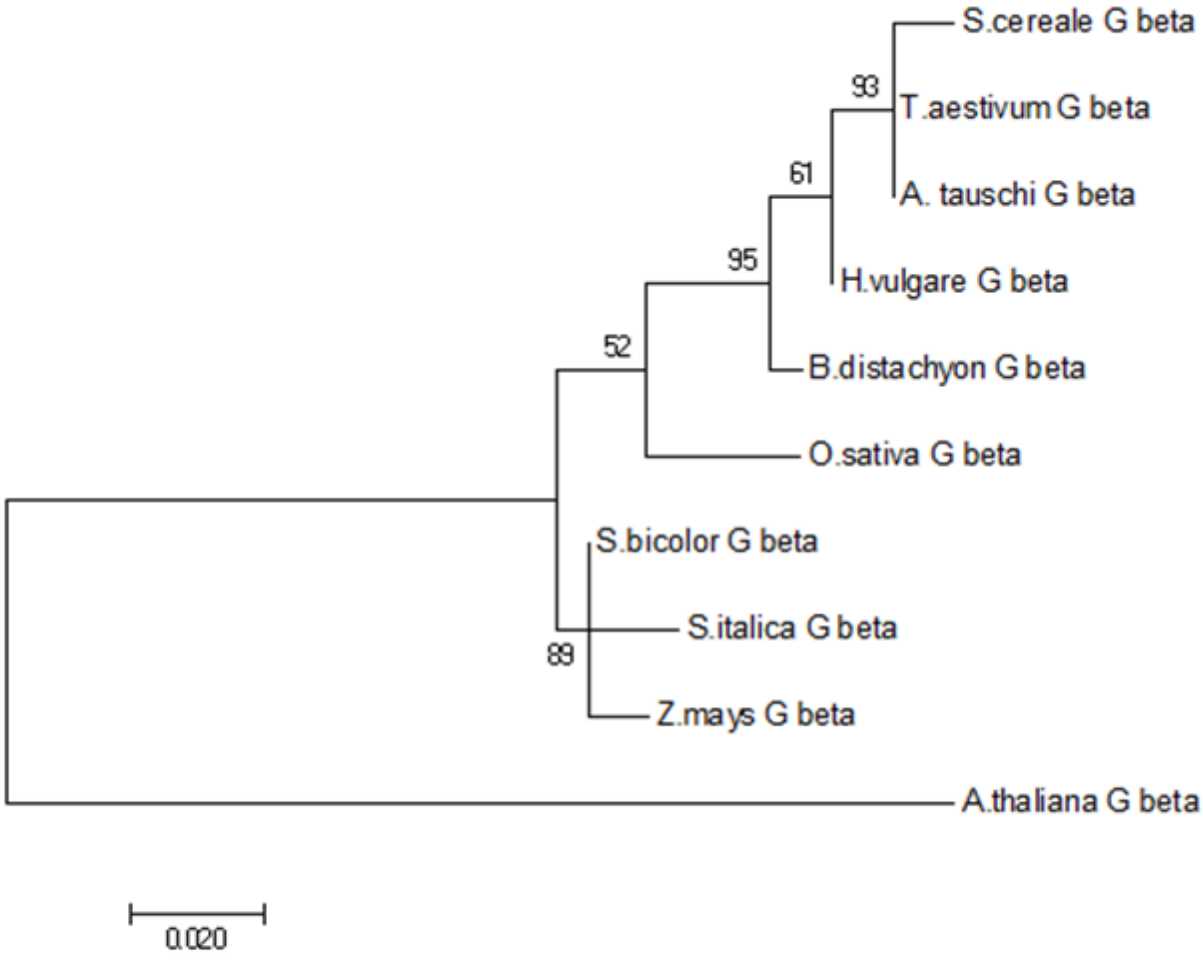
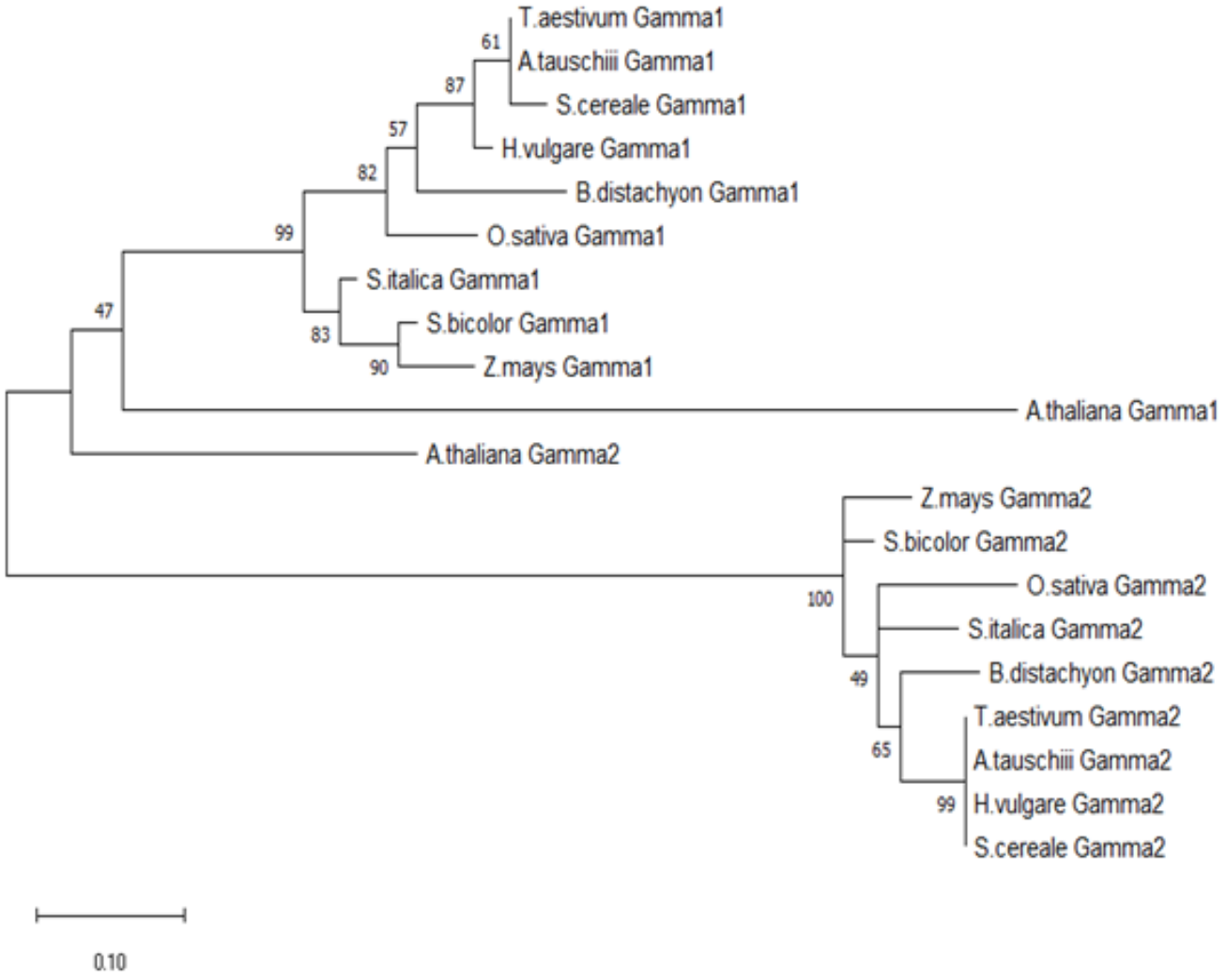

Supplementary Figure S2.4. Multiple sequence alignment of G protein γ3, γ4 and γ5 subunits from *Triticum aestivum* (A, B and *D homeologs), mon*ocot species *Aegilops tauschii, Hordeum vulgare, Secale cereale, Brachypodium distachyon, Setaria italica, Oryza sativa, Zea mays ,Sorghum bicolor* and dicot *Arabidopsis thaliana* by clustal omega. The conserved DPLL motif that forms hydrophobic contact with Gβ is shown.



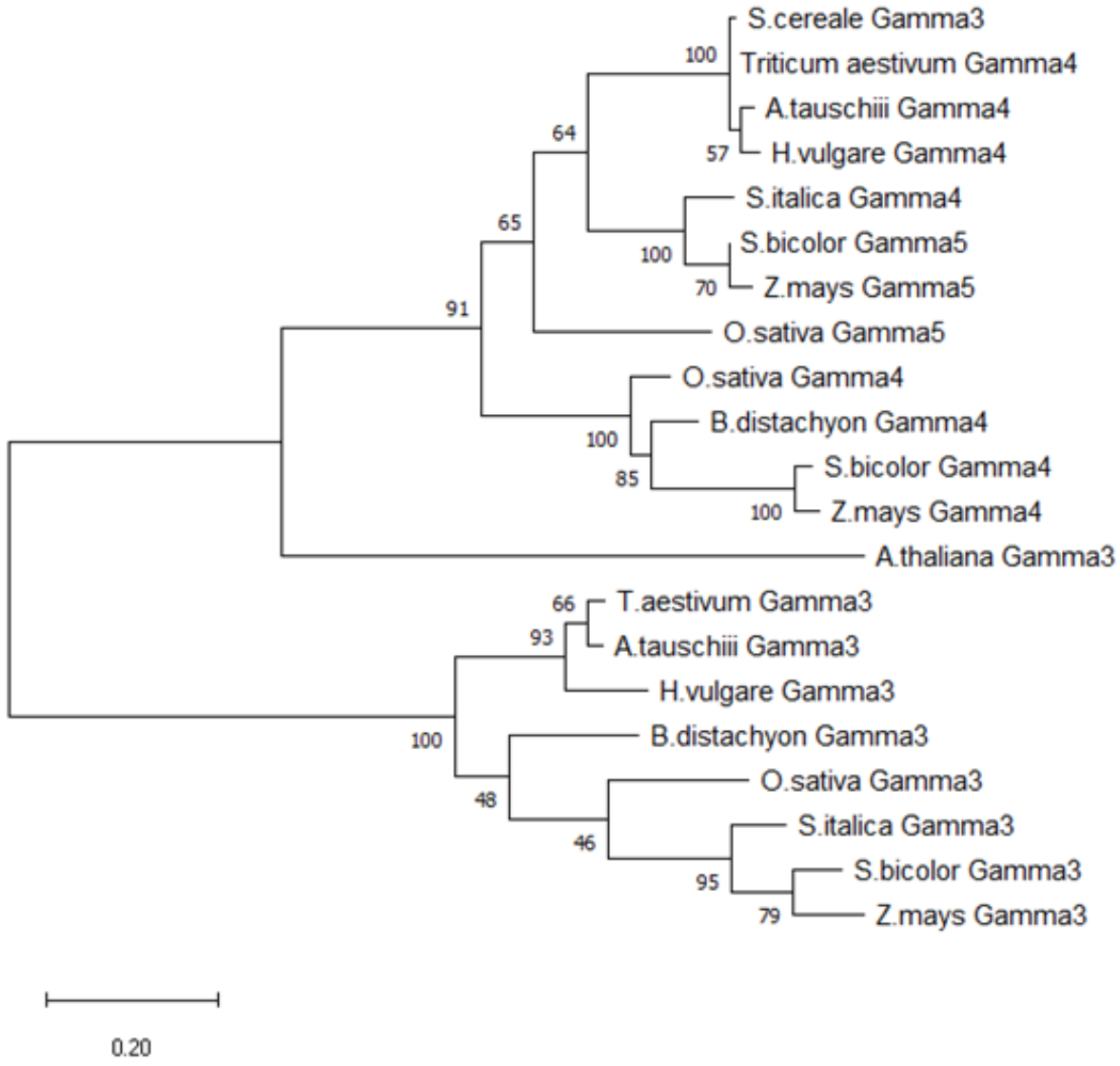
Supplementary Figure S2.5. Molecular phylogenetic analysis of G protein α subunits in ten species. The evolutionary history was inferred by using the Maximum Likelihood method based on the Whelan And Goldman model. The tree with the highest log likelihood (-1581.7231) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7189)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 amino acid sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 277 positions in the final dataset.



Supplementary Figure S2.6. Molecular phylogenetic analysis of G protein β subunits in ten species. The evolutionary history was inferred by using the Maximum Likelihood method based on the Whelan And Goldman model. The tree with the highest log likelihood (-1007.5736) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6463)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 amino acid sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 219 positions in the final dataset.



Supplementary Figure S2.7. Molecular phylogenetic analysis of Gγ1 and Gγ2 protein subunits in ten species. The evolutionary history was inferred by using the Maximum Likelihood method and Whelan And Goldman model. The tree with the highest log likelihood (-1030.63) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 2.6948)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 20 amino acid sequences. There were a total of 86 positions in the final dataset.



Supplementary Figure S2.8. Molecular phylogenetic analysis of Gγ3, Gγ4 and Gγ5 protein subunits in ten species. The evolutionary history was inferred by using the Maximum Likelihood method and Whelan And Goldman model. The tree with the highest log likelihood (-2179.81) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.5231)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 21 amino acid sequences. There were a total of 140 positions in the final dataset.

Supplementary Table S2.1. Details of the RNA-Seq datasets the raw reads used in expression analysis of G protein gene families in *Triticum aestivum*

Supplementary Table S2.1-A. Details of the RNA-Seq datasets used gene expression analysis of *Triticum aestivum*

Details about data	ENA SRA id	Data Source	Total Reads	Replicates
Five tissue types				
a) Fruit whole plant ripening stage	ERR414721_2, ERR414750_2	SRA database at NCBI	49053093, 30038860	2
b) Cotyledon emergence root	ERR424737_2, ERR424770_2	Pingault et al., 2005	45116709, 51833599	2
c) Leaf	ERR414749_2, ERR414763_2		47106971, 33655313	2
d) Stem	ERR414733_2, ERR414767_2		40953211, 55483346	2
e) Inflorescence	ERR414735_2, ERR414753_2		52120581, 45271806	2
Seventy one tissues Azhurnya wheat		eFP Browser at bar.utoronto.ca		3
Cold stress				
a) Control	SRR1460549, SRR1460550, SRR1460551	Array express	32616607, 77577791, 28872198	3
b) Cold stress	SRR1460552, SRR1460553, SRR1460554		40138740, 25425859, 19047190	3
Drought, heat and combined stress				
a) Control	SRR1542404_2, SRR1542405_2	SRA database	81155853, 75969741	2
b) Drought stress 1hr	SRR1542406_2, SRR1542407_2	at NCBI	68467921, 75864652	2
c) Drought stress 6hr	SRR1542408_2, SRR1542409_2		73614455, 73614455	2
d) Heat stress 1hr	SRR1542410_2, SRR1542411_2		66035008, 51618473	2
e) Heat stress 6hr	SRR1542412_2, SRR1542413_2		76623839, 67378274	2
f) Combined stress 1hr	SRR1542414_2,		53762767,	2

	SRR1542415_2		55585647	
g) Combined stress 6hr	SRR1542416_2, SRR1542417_2		53901424, 56318014	2
F. graminearum infection	**NIL51**			
a) Mock 24hr	ERR1201806_2, ERR1201807_2, ERR1201808_2	Array Express	22984326, 27832536, 20840051	3
b) *F. graminearum 24hr*	ERR1201788_2, ERR1201789_2, ERR1201790_2		27578418, 34713400, 31343655	3
c) Mock 48hr	ERR1201815_2, ERR1201816_2, ERR1201817_2		34787468, 20013677, 23404211	3
d) *F. graminearum 48hr*	ERR1201797_2, ERR1201798_2 ERR1201799_2		24045575, 32710023, 25984652	3
	NIL38			
e) Mock 24hr	ERR1201770_2, ERR1201771_2, ERR1201772_2	Array Express	25917089, 29732769, 24822761	3
f) *F. graminearum 24hr*	ERR1201752_2, ERR1201753_2, ERR1201754_2		25747899, 32609188, 27776756	3
g) Mock 48hr	ERR1201779_2, ERR1201780_2, ERR1201781_2		25760916, 38829518, 25218505	3
h) *F. graminearum 48hr*	ERR1201761_2, ERR1201762_2, ERR1201763_2		22760086, 37353756, 26837655	3

Note: The data sources, number of replicates, total reads in raw data and SRA data id used for downloads from European Nucleotide Archive ENA EMBL-EBI are given above.
Array Express (https://www.ebi.ac.uk/arrayexpress/) and SRA database at NCBI (https://www.ncbi.nlm.nih.gov/sra) were searched for the collection of data. The above data include five tissue type data from Pingault et al. 2015; seventy one tissues data from eFP browser from Ramírez-González et al. 2018 and Winter et al., 2007 and cold stress data from Li et al., 2015.

Supplementary Table S2.1-B. Details of the raw reads for G protein gene families after alignment to RNA-Seq datasets

ENA SRA id for replicates	Raw reads hits obtained after alignment in RNA-Seq for *Triticum aestivum* G protein gene families																	
	GA1			GB			Gγ1			Gγ2			Gγ3			Gγ4		
	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D
ERR414721_2	3	4	32	85	57	41	19	18	74	193	65	90	16	26	6	5	3	7
ERR414750_2	2	5	14	51	57	19	10	17	32	76	57	48	12	13	5	2	1	2
ERR424737_2	23	24	79	91	136	95	203	124	126	108	83	84	32	60	34	23	25	23
ERR424770_2	22	9	49	103	167	106	178	123	143	100	146	87	33	66	39	22	16	23
ERR414749_2	3	5	21	58	62	68	1	1	5	134	114	110	9	20	18	2	21	4
ERR414763_2	3	2	18	47	39	41	0	1	4	87	65	65	1	6	1	0	15	3
ERR414733_2	21	18	70	118	125	94	24	10	19	92	91	80	24	31	24	118	189	165
ERR414767_2	53	14	82	107	210	159	37	28	11	146	98	94	13	44	36	102	215	171
ERR414735_2	35	36	105	115	166	117	10	10	10	62	50	49	53	53	53	199	167	211
ERR414753_2	48	25	72	118	144	119	5	4	14	60	86	55	36	47	34	149	114	116
SRR1460549_2	19	4	47	37	69	46	0	3	4	0	66	144	6	10	4	0	0	1
SRR1460550_2	29	7	75	51	109	87	6	12	6	0	128	223	2	18	7	2	8	7
SRR1460551_2	17	3	28	24	51	35	1	4	2	0	47	91	1	7	3	1	2	1
SRR1460552_2	7	8	59	19	59	38	1	5	20	0	74	104	0	3	1	2	2	3
SRR1460553_2	2	4	42	10	24	33	3	3	13	0	33	53	0	3	1	0	1	2
SRR1460554_2	3	2	23	8	24	19	0	2	8	0	37	44	0	0	0	0	1	1

SRR1542404_2	37	20	66	93	138	105	1	4	3	59	100	81	2	2	14	7	12	10
SRR1542405_2	33	8	42	46	103	55	0	2	3	58	75	51	4	7	6	5	7	7
SRR1542406_2	11	4	21	46	64	47	3	3	2	46	58	48	3	3	5	4	6	8
SRR1542407_2	25	7	52	64	108	90	2	5	6	78	77	88	4	2	9	7	17	10
SRR1542408_2	18	4	34	45	37	62	2	6	8	33	54	51	4	7	10	10	6	4
SRR1542409_2	29	9	51	49	93	63	1	3	5	41	53	53	2	2	7	15	6	8
ERR1201790_2	2	3	9	24	41	28	0	1	2	25	40	34	0	1	1	0	2	1
SRR1542411_2	1	5	3	16	17	21	0	0	1	26	50	28	0	0	1	2	3	2
SRR1542412_2	6	3	55	89	111	64	2	1	3	101	152	134	3	3	7	0	1	0
SRR1542413_2	9	3	54	73	108	48	0	1	1	72	116	16	0	1	5	1	3	1
SRR1542414_2	2	1	17	15	22	8	1	1	1	16	31	25	1	0	2	0	1	1
SRR1542415_2	2	2	12	27	21	19	0	3	0	23	24	22	0	0	0	0	0	0
SRR1542416_2	5	3	39	51	92	59	0	0	4	57	114	99	1	0	1	1	5	2
SRR1542417_2	5	0	35	47	73	49	0	0	0	70	118	84	1	1	2	2	9	3
ERR1201806_2	18	10	17	31	49	31	4	3	2	6	15	14	2	51	24	23	30	40
ERR1201807_2	10	17	11	38	65	40	2	0	5	13	17	18	3	33	19	31	20	39
ERR1201808_2	14	12	18	36	41	30	2	1	0	15	18	16	0	29	8	25	18	39
ERR1201788_2	24	9	22	34	46	49	1	2	9	5	18	11	7	44	34	25	21	45
ERR1201789_2	18	21	16	55	64	76	3	4	0	23	30	12	4	52	45	25	32	44
ERR1201790_2	11	9	32	41	67	38	4	1	0	22	22	36	2	49	35	27	18	43
ERR1201815_2	17	15	30	53	73	61	5	0	5	17	9	21	7	63	21	31	46	59
ERR1201816_2	5	14	19	27	53	31	2	2	2	14	17	20	2	29	9	19	19	40

ERR1201817_2	12	21	35	38	65	45	0	1	0	12	12	17	0	45	25	20	24	31
ERR1201797_2	11	14	22	34	53	43	6	0	3	11	12	14	2	53	26	15	13	24
ERR1201798_2	17	15	31	50	91	51	12	4	6	18	21	34	1	86	59	17	21	40
ERR1201799_2	11	20	32	39	58	33	7	1	4	22	20	30	2	45	27	17	13	27
ERR1201770_2	20	14	14	28	77	47	2	3	1	20	11	13	4	52	28	19	23	34
ERR1201771_2	14	28	27	46	64	39	6	0	2	7	11	15	0	46	23	30	30	37
ERR1201772_2	9	5	21	36	64	40	2	2	1	16	21	23	1	60	27	20	24	36
ERR1201752_2	16	7	25	25	47	31	7	2	1	10	14	16	1	67	38	22	22	29
ERR1201753_2	25	13	31	48	67	42	3	1	2	14	18	28	2	40	24	26	19	36
ERR1201754_2	19	19	30	44	75	46	7	2	6	13	20	14	5	48	30	14	16	21
ERR1201779_2	15	15	28	32	64	43	1	3	0	23	9	19	0	49	24	12	12	25
ERR1201780_2	29	9	34	56	91	61	1	0	4	22	33	24	6	67	39	38	34	31
ERR1201781_2	12	11	4	44	63	39	2	0	1	10	14	16	4	59	32	22	25	30
ERR1201761_2	31	7	16	30	57	40	3	2	5	24	27	14	3	40	38	8	17	16
ERR1201762_2	13	23	34	62	99	62	13	0	1	13	32	36	5	87	74	20	17	54
ERR1201763_2	16	8	28	46	61	33	2	0	18	7	27	18	6	51	27	14	10	27

Note: The table above denotes the hits for each homeologous copies of G protein gene family members in *T. aestivum* in the RNA-Seq datasets.

Supplementary Table S2.2. Sequences of *Triticum aestivum* G protein gene family members used for alignment in RNA-Seq analysis

Gene name-copy	Region used for RNA-Seq data alignment	Length in nt
GA1-A	Stop codon+3' UTR region	224
GA1-B	Stop codon+3' UTR region	314
GA1-D	Stop codon+3' UTR region	217
Gβ-A	Stop codon+3' UTR region	344
Gβ-B	Stop codon+3' UTR region	357
Gβ-D	Stop codon+3' UTR region	311
Gγ1-A	Coding region+3' UTR	605
Gγ1-B	Coding region+3' UTR	619
Gγ1-D	Coding region+3' UTR	602
Gγ2-A	Stop codon+3' UTR region	340
Gγ2-B	Stop codon+3' UTR region	288
Gγ2-D	Stop codon+3' UTR region	327
Gγ3-A	Coding region+3' UTR	729
Gγ3-4A	Coding region+3' UTR	820
Gγ3-D	Coding region+3' UTR	755
Gγ4-A	Coding region+3' UTR	1126
Gγ4-B	Coding region+3' UTR	1062
Gγ4-D	Coding region+3' UTR	1085

Note: The details for the regions and length in nucleotides of G protein gene families in *T. aestivum* used in the alignment with RNA-Seq reads are given. In case of more similarity in the coding regions 3'UTR regions were used to distinguish between homeologous copies.

Supplementary Table S2.3. The identifiers for *Triticum aestivum* heterotrimeric G protein gene family members on 61K wheat Affymetrix microarray

Gene name	Hit on 61K wheat Affymetrix microarray
GA1	TaAffx.36446.1.S1_at
Gβ	Ta.14351.1.S1_at
Gγ1	TaAffx.40606.1.S1_at
Gγ2	TaAffx.84171.1.S1_at
Gγ3	TaAffx.106223.1.S1_at
Gγ4	Ta.7123.2.S1_at

Supplementary Table S2.4. *Triticum aestivum* nucleotide and protein sequences

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>Ta-*GA1*-B cDNA sequence
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>Ta-*Gβ*-D cDNA sequence
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>Ta-*Gβ*-D coding sequence
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>Ta-*Gβ*-D protein sequence
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>Ta-*Gγ1*-A cDNA sequence
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>Ta-*Gγ1*-A protein sequence
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>Ta-*Gγ1*-B cDNA sequence
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>Ta-*Gγ1*-B coding sequence
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>Ta-Gγ1-5B protein sequence
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>Ta-*Gγ1*-D cDNA sequence
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>Ta-*Gγ1*-D protein sequence
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>Ta-*Gγ2*-A cDNA sequence
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>Ta-*Gγ2*-B coding sequence
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>Ta-*Gγ2*-D cDNA sequence
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>Ta-*Gγ2*-D protein sequence
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>Ta-*Gγ3*-A cDNA sequence
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>Ta-*Gγ3*-A protein sequence
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>Ta-*Gγ3*-4A cDNA sequence
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>Ta-*Gγ3*-4A coding sequence
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>Ta-*Gγ3*-4A protein sequence
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>Ta-*Gγ3*-D cDNA sequence
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GGCGCTGTGGATTTGCCTGGTTTTACTTGCCACAGCGATGGATCCCTTGTGCTTTCGTCCTTGCTATAGTCTCCTATGTTGTAATTA
AAGGTTTGGAGATAACTGCAGAATGAACGGTTCTTCTCACT

>Ta-*Gγ3*-D coding sequence

ATGGCGGCGCCCAGGCCCAAGTCCCCGCTCGACCCCTGCGGCCGCCACCGGCTGCAGCTCGCCGTCGACGCGCTCCACCGCCAGATC
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GCGTGTTTGAGCTGCTTCCCGTGGATCTGCGGCGGCGGGTGCTCCGCCGTCCAGCTCAAGGGGCCGAGCTGCTGCTGCGGATGCCCC
CGTTGCTGCGTGGGGAGCGGGGGCTGCGGCGGCGGACCCTCGTGTGGCTGCTCATGCTCCTGCGCCGGTTGCTCCTCCTCTTGCGCG
TGCCCTGCCTGCGCCGGCTGCGGCGCCGCGTGCTGCGGCGGTGCCCCTCGCCCTCGCTGCTGCCTGTGTTCATGA

>Ta-*Gγ3*-D protein sequence
MAAPRPKSPLDPCGRHRLQLAVDALHRQISFLEGEISSIEGLHAASICCKEVDEFIGKNA
DPFITISSEKGNADQSHRFPKKIRTRWACLSCFPWICGGGCSAVQLKGPSCCCGCPRCCV
GSGGCGGGPSCGCSCSCAGCSSSCACPACAGCGAACCGGAPRPRCCLCS

>Ta-*Gγ4*-A cDNA sequence
GCGCAGCGCGAGCTATATGGAGGGACTCCTCGCCTCCGCCTTCATTTCCACCACCTGCTCTGCTCTGCTCCGCTCCGCTCCTCCCCA
GCCCACGCCCCCGCCTCCATCCCCTCGCGTCGCACGCACGCGCTCGCCTGCGCCGCTCAGATTCCTCGCATCACCGCCGGCCAGCGC
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GCCGAGGTACCCGGACATGTGCGGCCGCCGGCGCCTGCAGCTGGAGGTGCAGATCCTTGACCGCGAGCTCACGTTCCTCAAGGACGA
GCTACATTTACTTGAAGGGGCTCAACCGGTCTCACGTTCTGGTTGCTTGAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGACCC
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CGGCGACTGCAAGCCAAGCTGCAGCTGCTGCGGCGAGCAGTGCTGCAGCTGCGCGGGCTGCTCCTGCCCTCGATGCACAGGGGGCTG
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AGTAGCCAAGTTATATATATTGGTGATGCACA

>Ta-*Gγ4*-A coding sequence
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TCTGGTTGCTTGAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGACCCACTAATACCAATTAACAAAAGGAAGCACCGGTCCTGC
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TGCGGCGAGTGCAAGCCGGAGTGCGGCTCATGTTCCGGCGGCGGCTGCTGCGGCGACTGCAAGCCAAGCTGCAGCTGCTGCGGCGAG
CAGTGCTGCAGCTGCGCGGGCTGCTCCTGCCCTCGATGCACAGGGGGCTGCCTCAGCTGCTTCAAGCTCCCCAAATGCTCCTGCGCG
CGGTGCTTCAACTGCCAGTCGTCCTGCTGCAAGGGGCAGCCGTCGTGCTTCAGGTGCCAGTCGTCGTGCTGCGACAAGGGGGGCTGC
TGCAGCGGCGGGTCGTGCCTGAGCTGCCCGAAGCCGTCGTGCCCCGAGTGCTCCTGCGGGTGCGTGTGGTCGTGCAAAAACTGTACA
GACGGATGCCGATGCGCCCGGTGCTGTGCTGGCGGGTGCCTGTGCTAA

>Ta-*Gγ4*-A coding sequence
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DCSCCEPNCSCCSPNCCSCSCFKIPPCCKPSCGCFDCCSCSCSKPQCCSGGCNLCGECKP
ECGSCSGGGCCGDCKPSCSCCGEQCCSCAGCSCPRCTGGCLSCFKLPKCSCARCFNCQSS
CCKGQPSCFRCQSSCCDKGGCCSGGSCLSCPKPSCPECSCGCVWSCKNCTDGCRCARCCA
GGCLC

>Ta-*Gγ4*-B cDNA sequence
GCGCAGCGCGAGCTATATGGAGAGACTCCTCGCCTCCGCCTTCATTTCACTTCCACCACCTGCTCTACTCTGCTCTGCTCCTCCCCA
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TCGCCGCCGAGGTACCCGGACATGTGCGGTCGCCGGCGCCTGCAGCTGGAGGTGCAGATCCTTGACCGCGAGCTCACGTTCCTCAAG
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GCTGTTTAAGCCTCTGTTTGGGTTCGTTCGGGGCCTTGACGTATGGTGGTGTTTCACATGTAAAGAAATAACTTGACCTCCGGATCA
CAAGGCGGAGTGAAGTAGCTCTAGTATCAGTAGCCAAGTTATATATATTGGTGATGCACATTGTCAACCATTTTACAGGGATTTTCT
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>Ta-*Gγ4*-B coding sequence
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TCTGGTTGCTTGAAAGAGGTAAACGAGTTTGTTGGTACAAAACAAGACCCGCTAATACCAATTAACAAAAGGAAGCACCGGTCCTGC
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TGCGTGTGGTCGTGCAAAAACTGTACAGACGGATGCCGATGCGCCCGGTGCTGTGCTGGCGGGTGCCTGTGTTAA

>Ta-*Gγ4*-B protein sequence
MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPVSRSG
CLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASWLCCSCQCLPTCKRPRCF
DCSCCEPNCSCCSLNCCSCFSIPSCCKPSCGCFECCSCSKPQCCSSGCNPCGECKPECGS
CSGGGCCGEQCCSCPRCTGCFSCFKVPKCSCAQCFNCQSSCCKGQPSCFRCQSSCCDKGG
CCSGGSCLSCPKPSCPECSCGCVWSCKNCTDGCRCARCCAGGCLC

>Ta-*Gγ4*-D cDNA sequence
GCGCAGCGCGAGCTATATGGAGAGACTCCTCGCCTCCGCCTTCATTTCATTTCCACCACCTGCTCTACTCTGCTCTGCTCCTCCCCA
GCCCACGCCCCCGCCCCCGCCCCCGTCCCCCGCCTCCATCTCCTCGCATCGCACGCACGCACGCACGCACGCACTCACCTGCGCTGC
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CCGTTCGGGGCCTTGACATACGGTGGTGTTTCACATGTATGTAAAGAAATAACTTGACCTCCGGATCACAAGGCGGAGTGAGTAGCT
CTAGTATCAGTAGCCAAGTTATATATATTGGTGATGCACATTGTCAACCATTTTACAGGGATTTTATTTTTATTTTTTATTCGGGA

>Ta-*Gγ4*-D coding sequence
ATGGGGGAGGGCGCGGTGGTGGTGCTGGAGGCGCCCAAGCCCAGGTCGCCGCCGAGGTACCCGGACATGTGCGGCCGCCGGCGCCTG
CAGCTGGAGGTGCAGATCCTTGACCGCGAGCTCACGTTCCTCAAGGACGAGCTACATTTACTTGAAGGGGCTCAACCAGTCTCACGT
TCTGGTTGCTTGAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGACCCGCTAATACCAATTAACAAAAGGAAGCACCGGTCCTGC

CGTCTTTACTGGTGGATCAGATCAAAACTGTGCATATGTGCTTCATGGCTGTGCTGCTCCTGCCAATGCCTACCAACTTGCAAAAGA
CCAATGTGCTTGGACTGTTCATGCTGCAAACCAAATTGCTCATGCTGCAGCCCAAACTGCTGCAGCTGCTTCAAGATCCCTTCATGC
TGCAAACCAAGCTGCGGCTGCTTCGAGTGCTGCAGCTGCAGCAAGCCACAGTGCTGCAGCAGCGGCTGTAACCCTTGCGGCGAGTGC
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CTGAGCTGCCCGAAGCCGTCGTGCCCCGAGTGCTCCTGCGGGTGCGTGTGGTCGTGCAAAAACTGTACAGACGGATGCCGATGCGCC
CGGTGCTGTGCTGGCGGGTGCCTGTGTTAA

>Ta-*Gγ4*-D protein sequence
MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPVSRSG
CLKEVNEFVGTKQDPLIPINKRKHRSCRLYWWIRSKLCICASWLCCSCQCLPTCKRPMCL
DCSCCKPNCSCCSPNCCSCFKIPSCCKPSCGCFECCSCSKPQCCSSGCNPCGECKPECGS
CSGGGGCCGDCKPSCSCCGEQCCSCAGCSCPRCTGGCFKLPKCSCARCFNCQSSCCKGQP
SCFRCQSSCCDKGGCCSGGSCLSCPKPSCPECSCGCVWSCKNCTDGCRCARCCAGGCLC

Supplementary Table S2.5. Identifiers for *Triticum aestivum* G protein gene family members at different databases

Gene	Chr	aa length	Start codon[a]	Alignment	TSA identifiers	EST identifiers
GA1-A	7AS	385	242753	(+/-)	HAAB01085718.1	CJ808857.1
GA1-B	1BL	367	687089859	(+/-)	JV870574.1	CJ675275.1
GA1-D	7DS	382	208597	(+/-)	GFFI01019335.1	CJ933261.1
Gβ-A	4AS	380	595555285	(+/-)	JP206703.1	CJ942954.1
Gβ-B	4BS	380	14113660	(+/-)	JP888677.1	CJ625484.1
Gβ-D	4DS	380	7838729	(+/+)	JV890233.1	CJ838192.1
Gγ1-A	5AL	98	575522382	(+/+)	JP925789.1	BJ285419.1
Gγ1-B	5BL	98	559678294	(+/+)	JP925791.1	ND
Gγ1-D	5DL	97	457624479	(+/+)	JP925790.1	CD891132.1
Gγ2-A	6AS	141	76625637	(+/+)	JP905142.1	CJ781741.1
Gγ2-B	6BS	146	133841813	(+/+)	JV852436.1	CJ900184.1
Gγ2-D	6DS	145	60131687	(+/+)	JV845957.1	CJ903178.1
Gγ3-A	7AS	169	7600093	(+/+)	GFFI01078888.1	ND
Gγ3-4A	4AL	170	733693637	(+/-)	GFFI01062727.1	ND
Gγ3-D	7DS	169	6485486	(+/+)	GFFI01069178.1	ND
Gγ4-A	5AL	305	430491028	(+/+)	JV837175.1,	BE499413.1
Gγ4-B	5BL	285	378518025	(+/-)	GFFI01047686.1	BJ315809.1,
Gγ4-D	5DL	299	326126873	(+/-)	GFFI01051477.1	CJ559675.1

Note: Chr denotes chromosome location for homeolgous gene copy. Start codon is the position of start codon at IWGSC RefSeqv 1.0. TSA and EST identifiers are the 99-100% hits at TSA and EST databases at NCBI.

Supplementary Table S2.6. Identifiers for genes encoding G proteins in monocot species

	Identifiers for genes encoding Gα proteins in monocot species in database			
Species	**NR**	**EST**	**TSA**	**PlantGDB**
H. vulgare	AF267485.2	EX582377.1	GGDJ01081284.1	gnl|HVcdna|11245654
B. distachyon	XM_003564993.3	GT825309.1	GFJC01117974.1	Bradi2g60350.1
S cereale	NA	NA	GCJW01013911.1	NA
S. italica	XM_004963062.3	NA	NA	Si022288m
S. bicolor	EU069505.1	CD224439.1	NA	Sb01g045320.1
Z. mays	EU969441.1	EE044851.2	NA	gnl|ZMcdna|195644181
A. tauschii	XM_020335686.1	NA	IAAO01017775.1	NA
	Identifiers for genes encoding Gβ proteins in monocot species			
H. vulgare	AK251844.1|	DK654503.1	IAAY01048238.1	NA
B. distachyon	XM_003561394.3	GT852640.1	GFJC01014458.1	Bradi1g12820.1
S cereale	NA	NA	GCJW01028980.1	NA
S. italica	XM_004982159.3	NA	GBYO01011226.1	Si036161m
S. bicolor	XM_002466646.1	CF430264.1	NA	Sb01g012370.1
Z. mays	EU12233.1	DV504210.1	NA	gnl|ZMcdna|557695
A. tauschii	XM_020325500.1	NA	IAAU01026447.1	NA
	Identifiers for genes encoding Gγ1 proteins in monocot species			
H. vulgare	AK359503.1	DK589354.1	GGCP01015868.1	NA
B. distachyon	XM_003559606.3	NA	GFJA01145038.1 (P)	Bradi1g14140.1
S cereale	NA	NA	GCJW01019647.1	NA
S. italica	XM_004982313.1	NA	NA	Si038059m
S. bicolor	XM_002464159.1	NA	NA	Sb01g014060.1
Z. mays	EU971644.1	EE680754.1	NA	gnl|ZMcdna|195648588
A. tauschii	XM_020345933.1	NA	IAAR01053401.1	NA

	Identifiers for genes encoding Gγ2 proteins in monocot species in databases			
Species	**NR**	**EST**	**TSA**	**PlantGDB**
H. vulgare	AK367089.1	DK635920.1	IAAY01026028.1	NA
B. distachyon	AK433245.1	HX811213.1	GFJA01116531.1	Bradi3g03350.1
S cereale	NA	NA	GCJW01024007.1	NA
S. italica	XM_004951888.3	NA	GBYO01012724.1	Si019618m
S. bicolor	XM_002451466.1	BG240019.1	NA	Sb04g003060.1
Z. mays	EU972438.1	CF637682.1	NA	gnl|ZMcdna|195650176
A. tauschii	XM_020321987.1	NA	IAAS01050579.1	NA
	Identifiers for genes encoding Gγ3 proteins in monocot species			
H. vulgare	NA	AV919502.1	GGDK01004430.1	NA
B. distachyon	XM_014896471.1	NA	NA	Bradi1g60176.1
S cereale	NA	NA	NA	NA
S. italica	XM_004984004.3	NA	NA	Si039839m

S. bicolor	XM_002465107.1	BI098087.1	NA	Sb01g032830.1
Z. mays	FJ797616.1	FL213580.1	NA	gnl|ZMcdna|268321221
A. tauschii	XM_020343714.1	NA	IAAR01037585.1	NA
	Identifiers for genes encoding Gγ4 proteins in monocot species			
H. vulgare	FJ039903.1	AV916432.1	GGDK01054667.1	NA
B. distachyon	XM_003572220.3	HX819832.1	GFJC01017681.1	Bradi3g37595.1
S cereale	NA	NA	GCJW01029735.1	NA
S. italica	XM_004956882.2	NA	NA	Si033308m
S. bicolor	XM_002444424.1	CN137908.1	NA	Sb07g022330.1
Z. mays	EU976637.1	DV622155.1	NA	gnl|ZMcdna|195658574
A. tauschii	XM_020298538.1	NA	IAAT01038459.1	NA
	Identifiers for genes encoding Gγ5 proteins in monocot species			
S. bicolor	XM_002460230.1	BG487595.1	NA	Sb02g025860.1
Z. mays	EU972808.1	FL432535.1	NA	gnl|ZMcdna|195650916

Supplementary Table S2.7. Sequences for G protein gene family members in monocot species and Arabidopsis.

>*A.thaliana_Gα* coding sequence
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CAAGAAGCAAAGGCTGAAAAGCATATTCGGAAGCTTTTGCTACTTGGTGCTGGGGAATCTGGAAAATCTACAATTTTTAAGCAGATA
AAACTTCTATTCCAAACGGGATTTGATGAAGGAGAACTAAAGAGCTATGTTCCAGTCATTCATGCCAATGTCTATCAGACTATAAAA
TTATTGCATGATGGAACAAAGGAGTTTGCTCAAAATGAAACAGATTCTGCTAAATATATGTTATCTTCTGAAAGTATTGCAATTGGG
GAGAAACTATCTGAGATTGGTGGTAGGTTAGACTATCCACGTCTTACCAAGGACATCGCTGAGGGAATAGAAACACTATGGAAGGAT
CCTGCAATTCAGGAAACTTGTGCTCGTGGTAATGAGCTTCAGGTTCCTGATTGTACGAAATATCTGATGGAGAACTTGAAGAGACTA
TCAGATATAAATTATATTCCAACTAAGGAGGATGTACTTTATGCAAGAGTTCGCACAACTGGTGTCGTGGAAATACAGTTCAGCCCT
GTGGGAGAGAATAAAAAAAGTGGTGAAGTGTACCGATTGTTTGACGTGGGTGGACAGAGAAATGAGAGGAGGAAATGGATTCATCTG
TTTGAAGGTGTAACAGCTGTGATATTTTGTGCTGCCATCAGCGAGTACGACCAAACGCTCTTTGAGGACGAGCAGAAAAACAGGATG
ATGGAGACCAAGGAATTATTCGACTGGGTCCTGAAACAACCCTGTTTTGAGAAAACATCCTTCATGCTGTTCTTGAACAAGTTCGAC
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ATTGAGCATGCATACGAGTTTGTGAAGAAGAAGTTTGAGGAGTTATATTACCAGAACACGGCGCCGGATAGAGTGGACAGGGTATTC
AAAATCTACAGGACGACGGCTTTGGACCAGAAGCTTGTAAAGAAAACGTTCAAGCTCGTAGATGAGACACTAAGAAGGAGAAATTTA
CTGGAGGCTGGCCTTTTATGA

>*A.thaliana_Gα* translation
MGLLCSRSRHHTEDTDENTQAAEIERRIEQEAKAEKHIRKLLLLGAGESGKSTIFKQIKLLFQTGFDEGELKSYVPVIHANVYQTIK
LLHDGTKEFAQNETDSAKYMLSSESIAIGEKLSEIGGRLDYPRLTKDIAEGIETLWKDPAIQETCARGNELQVPDCTKYLMENLKRL
SDINYIPTKEDVLYARVRTTGVVEIQFSPVGENKKSGEVYRLFDVGGQRNERRKWIHLFEGVTAVIFCAAISEYDQTLFEDEQKNRM
METKELFDWVLKQPCFEKTSFMLFLNKFDIFEKKVLDVPLNVCEWFRDYQPVSSGKQEIEHAYEFVKKKFEELYYQNTAPDRVDRVF
KIYRTTALDQKLVKKTFKLVDETLRRRNLLEAGLL

>*A.thaliana_Gβ* coding sequence
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CAGCTCCTCGATACCGATGTGGCGAGGTATTCAGCGGCGCAAGGACGTACTCGGGTGAGCTTCGGAGCAACGGATCTGGTTTGTTGT
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AGATTAATCGTGTGGAATGCTCTAACGAGTCAGAAAACTCATGCTATTAAACTCCCTTGTGCATGGGTTATGACATGTGCTTTCTCT
CCAAATGGTCAGTCGGTTGCGTGTGGTGGATTAGACAGTGTATGTTCTATCTTTAGCCTTAGCTCAACGGCGGACAAGGATGGAACT
GTACCGGTTTCAAGAATGCTCACTGGTCACAGGGGATATGTTTCGTGCTGTCAGTATGTCCCAAATGAGGATGCCCACCTTATCACC
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GACACTCGTGCTGCAAGCCGAGCAGTGCGTACCTTTCATGGTCACGAGGGAGATGTTAATACGGTCAAGTTCTTTCCGGATGGGTAT
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TGA

>*A.thaliana_Gβ* translation
MSVSELKERHAVATETVNNLRDQLRQRRLQLLDTDVARYSAAQGRTRVSFGATDLVCCRTLQGHTGKVYSLDWTPERNRIVSASQDG
RLIVWNALTSQKTHAIKLPCAWVMTCAFSPNGQSVACGGLDSVCSIFSLSSTADKDGTVPVSRMLTGHRGYVSCCQYVPNEDAHLIT
SSGDQTCILWDVTTGLKTSVFGGEFQSGHTADVLSVSISGSNPNWFISGSCDSTARLWDTRAASRAVRTFHGHEGDVNTVKFFPDGY
RFGTGSDDGTCRLYDIRTGHQLQVYQPHGDGENGPVTSIAFSVSGRLLFAGYASNNTCYVWDTLLGEVVLDLGLQQDSHRNRISCLG
LSADGSALCTGSWDSNLKIWAFGGHRRVI

>*A.thaliana_Gγ*1 coding sequence
ATGCGAGAGGAAACTGTGGTTTACGAGCAGGAGGAGTCTGTTTCTCACGGCGGGGGCAAGCACAGGATCCTTGCAGAGCTTGCCCGC
GTTGAACAGGAAGTCGCTTTCTTGGAGAAAGAGTTGAAGGAGGTCGAGAACACAGATATTGTATCAACCGTGTGTGAGGAGCTGCTA
TCTGTCATCGAGAAAGGACCCGATCCTCTGTTGCCACTAACCAATGGACCTTTGAACTTAGGATGGGACCGGTGGTTTGAAGGACCA
AATGGAGGAGAAGGCTGCAGATGCTTAATACTTTGA

>*A.thaliana_Gγ*1 translation
MREETVVYEQEESVSHGGGKHRILAELARVEQEVAFLEKELKEVENTDIVSTVCEELLSVIEKGPDPLLPLTNGPLNLGWDRWFEGP
NGGEGCRCLIL

>*A.thaliana_Gγ*2 coding sequence
ATGGAAGCGGGTAGCTCCAATTCGTCGGGTCAGCTATCCGGGCGGGTCGTTGATACAAGAGGCAAACACAGGATTCAAGCTGAACTC
AAAAGGCTTGAACAAGAAGCTCGATTCTTAGAGGAAGAGCTGGAGCAGCTTGAGAAGATGGACAATGCATCAGCATCCTGCAAAGAG
TTCTTAGACAGTGTTGACAGCAAACCCGATCCTCTTCTTCCCGAAACAACAGGTCCGGTGAATGCGACATGGGATCAATGGTTCGAA
GGCCCTAAAGAAGCAAAACGATGTGGCTGCTCCATTCTTTGA

>*A.thaliana_Gγ*2 translation
MEAGSSNSSGQLSGRVVDTRGKHRIQAELKRLEQEARFLEEELEQLEKMDNASASCKEFLDSVDSKPDPLLPETTGPVNATWDQWFE
GPKEAKRCGCSIL

>*A.thaliana_Gγ*3 coding sequence
ATGTCTGCTCCTTCTGGCGGTGGCGAAGGAGGAGGAAAAGAATCAGCTGCTGGTGGAGTGAGTTCATCGTCTCTTGCTCCGTCGTCT
CTACCACCGCCTCGTCCTAAGTCTCCACCAGAGTATCCAGATTTGTACGGGAAACGCAGAGAGGCGGCGAGAGTTCAGATGCTCGAG
AGAGAGATTGGTTTTCTCGAGGGCGAAATTAAATTCATCGAAGGCGTACAACCGGCATCTAGATGCATCAAAGAAGTCTCTGATTTT
GTTGTTGCAAATTCTGACCCATTGATCCCTGCACAACGAAAAAGTCGAAGATCCTTCCGGTTCTGGAAGTGGCTCTGTGGCCCATGT
TTGAGCCTGGTGAGTTTCTGCTGTTGCTGCCAATCCAAATGTTCGTGCCATCTGAGGAAACCCAAGTGCTGCAACTGTACATCTTGC
AGCTGTATAGGGTCCAAATGCTGTGACGGGTCATGCTGCTCAAACATTTGTTGTTGCCCGAGACTAAGCTGCCCGAGCTGTTCATGC
TTCCGAGGTTGCTGGTGTTCTTGTCCGGACATGTCTTGCTGCATTCCCAGCTGTTTCCGCAGTTGCAGTTGCACTCGACCGTCGTGT
CTGAATAAAAAGAAGAGCTCATGCTGCAGCTGCAACTGCAAGATCAGATGGTCATCTTGTTTTAGTTGTCCCAAGGTACGACTTTGT
TCTTGTTGTTTTTGCAATTGTAAAAATCTATGTTCTAATCCTTGTTGTTTAGCTTTCTAA

>*A.thaliana_Gγ*3 translation
MSAPSGGGEGGGKESAAGGVSSSSLAPSSLPPPRPKSPPEYPDLYGKRREAARVQMLEREIGFLEGEIKFIEGVQPASRCIKEVSDF
VVANSDPLIPAQRKSRRSFRFWKWLCGPCLSLVSFCCCCQSKCSCHLRKPKCCNCTSCSCIGSKCCDGSCCSNICCCPRLSCPSCSC
FRGCWCSCPDMSCCIPSCFRSCSCTRPSCLNKKKSSCCSCNCKIRWSSCFSCPKVRLCSCCFCNCKNLCSNPCCLAF

>*A.tauschii_Gα* coding sequence
ATGGGCTCATCCTGCAGCAGACCTCACTCAGTAAATGAGGCAGACGCAGCTGACAACACAAGATCTGCAGACATCGACCGGCGGATT
CTTCAGGAGACAAAGGCGGACCAGCACATCCACAAGCTCTTGCTTCTTGGTGCCGGAGAATCAGGAAAGTCCACGATATTTAAACAG
ATCAAGCTTCTTTTCCGAACCGGCTTCGACGAGGCAGAACTCAAGGGCTATACGCCCGTCATCCATGCCAACGTGTTCCAGACAATC
AAAATACTATACGATGGAGCTAAAGAGCTTGCCCAAGTGGAACCCGAGTCTTCAAAATATGTGATATTACCCGATAATCAGGAGATT
GGAGAAAAACTATCAGAAATCGGAGGCAGGTTGGATTACCCGTCGCTTAACAAAGAACTCGTACAGGATGTAAGAAAATTATGGGAA
GATCAAGCCATTCAGGAAACTTACTCGTGTGGAAGTGTGCTGCAAGTTCCTGACTGTGCACACTACTTCATGGACAATTTGGACCGA
TTAGCTGAAGCAGATTACGTACCAACAAAGGAGGATGTGCTCCATGCAAGAGTGCGGACAAATGGGGTTGTAGAAATTCAATTTAGC
CCCCTTGGAGAGAGCAAAAGGGGCGGAGAGGTGTACAGGCTGTACGACGTAGGGGGTCAAAGGAATGAGAGAAGGAAGTGGATTCAT
CTCTTTGAAGGTGTTGATGCAGTTATCTTTTGTGCTGCCATTAGCGAGTACGATCAGTTGTTATTTGAGGACGAGACGCAGAACAGG
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GATGTGGAGCATGCCTATGAGTTCGTGAAGAAGAAGTTTGAGGAGGTCTACTTCCAGAGCAGCAAGCCCGAGCGTGTCGACCGGGTG
TTCAAGATCTACAGAACGACAGCGCTGGACCAGAAACTTGTAAAGAAGACGTTCAAGCTGATGGACGAGAGCATGAGACGCTCCCGG
GAAGGAACGGGGACGTGA

>*A.tauschii_Gα* translation
MGSSCSRPHSVNEADAADNTRSADIDRRILQETKADQHIHKLLLLGAGESGKSTIFKQIKLLFRTGFDEAELKGYTPVIHANVFQTI
KILYDGAKELAQVEPESSKYVILPDNQEIGEKLSEIGGRLDYPSLNKELVQDVRKLWEDQAIQETYSCGSVLQVPDCAHYFMDNLDR
LAEADYVPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAVIFCAAISEYDQLLFEDETQNR
MMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKVPLTVCEWFKDYEPIAPGKQDVEHAYEFVKKKFEEVYFQSSKPERVDRV
FKIYRTTALDQKLVKKTFKLMDESMRRSREGTGT

>*A.tauschiii_Gβ* coding
sequenceATGGCATCCGTGGCGGAGCTCAAGGAGAAGCACGCGGCGGCGACGGCCTCGGTCAACTCCCTCCGGGAGCGGCTCCGCC

AGCGCCGCCAGACGCTCCTCGACACCGACGTGGAGAAGTACTCCAAGGCGCAGGGGCGGACGGCGGTGAGCTTCAACCCCACGGATC
TGGTGTGCTGCCGCACGCTGCAGGGCCACAGCGGAAAGGTATATTCTCTGGATTGGACTCCTGAGAAGAACTGGATAGTTAGTGCCT
CACAAGATGGAAGACTAATTGTATGGAATGCTTTAACGAGTCAGAAAACACATGCCATAAAGCTACACTGTCCATGGGTGATGACAT
GTGCTTTTGCACCCAATGGTCAATCTGTTGCTTGCGGCGGTCTTGATAGTGCATGCTCTATATTCAACCTTAGCTCACAAGCAGACA
GAGATGGGAACATGCCAGTATCAAGAGTACTTACTGGACACAAAGGCTATGTTTCATCCTGTCAGTATGTCCCAGACCAGGAAACCC
GCTTGATTACAGGCTCAGGTGACCAAACGTGTGTCCTGTGGGATGTTACTACTGGCCAGAGGATATCAATCTTTGGAGGTGAATTTC
CATCAGGGCATACAGCTGATGTCTTAAGTCTGTCCATCAACTCGTTAAACACGAATATGTTTATCTCGGGTTCATGTGATACAACTG
TAAGGTTATGGGATCTCAGGATTGCAAGTCGGGCAGTTCGGACATATCATGGACATGAGGGCGACATTAACAGTGTCAAGTTTTTCC
CTGATGGTCAGAGGTTCGGTACTGGTTCAGATGATGGTACATGCAGATTATTTGACATGAGAACAGGGCATCAACTTCAAGTGTACA
ATCGGGAGCCCGATAGAAATGATAATGAGCTCCCCATCGTTACATCTGTCGCTTTCTCCATATCAGGAAGGCTTCTTTTTGCTGGAT
ACTCTAATGGTGACTGTTATGTGTGGGACACGCTTCTTGCCGAGATGGTGCTTAATTTGGGAACTCTCCAAAACTCTCACGAAGGCC
GTATAAGCTGCCTTGGGCTGTCATCCGATGGGAGTGCATTGTGTACAGGAAGCTGGGACAAAAATTTGAAGATCTGGGCTTTCAGTG
GACACCGCAAGATAGTCTGA

>*A.tauschii_Gβ* translation
MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLSSQADRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLI
TGSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASRAVRTYHGHEGDINSVKFFPDG
QRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPIVTSVAFSISGRLLFAGYSNGDCYVWDTLLAEMVLNLGTLQNSHEGRIS
CLGLSSDGSALCTGSWDKNLKIWAFSGHRKIV

>*A.tauschiii_Gγ*1 coding sequence
ATGCAGGTTCCGGGAGACGTCGGCGGGGGTGGAGGGGAAGCCGGGGACATGCGGGGCCGGCACCGGATCCAGGCCGAGCTCAAGAAG
CTCGAGCAAGAAGCGCGCTTCCTCGAGGAGGAACTTGAAGAGCTAAATAAGATGGATAAGGTGTCAACAGCACTGCAAGAGTTTGTA
GTAACAATTGAAAGCAAAGCAGACCCTCTACTTCCTGTAACTACTGGAGCTGCTTACCAGTCTTGGGACAGGTGGTTTGAAGGTCCG
CAGGATCTGCGTAGATGCAAATGCTGGTTTTTGTGA

>*A.tauschiii_Gγ*1 translation
MQVPGDVGGGGGEAGDMRGRHRIQAELKKLEQEARFLEEELEELNKMDKVSTALQEFVVTIESKADPLLPVTTGAAYQSWDRWFEGP
QDLRRCKCWFL

>*A.tauschiii_Gγ*2 coding sequence
ATGAGGGGGGAGGCCAACGGAGGGGACCGGCGGCCGCGGGACGAGGAGGGGGAGGGGGAGGAGGAGGAGGAGCCGCCGCAGCAGCAG
GAGGAGGAGAGGGCGGCGAGGCCGTCTTCTGAGCAGCAGCAGCCCGTTGCTGCGGAGGCGGCGGCGACGACGACGACGAGGAGCGTG
GGGTACGTGGGGAAGCACCGCCTCTCCGCCGCCATCCAGCGCCTCGACCAGGAGCTCCAGTCACTCCAGGATGAATTGAATGAGCTT
GAAACCATGGAACCTGCATCTGCGGCATGCCGGGAGGTGATCACAAGTACTGAAGGAAAACCTGACCCGCTTCTTCCAATCACAAGT
AGCCCGGAGAACTCTTCATGGGACAGGTGGTTCCAGCGCGTGCGAAGCTCTCGCAGCAACAAATGGTGGCAATCCAAGGGCTCTGAT
TTTGCCTAG

>*A.tauschiii_Gγ*2 translation
MRGEANGGDRRPRDEEGEGEEEEEPPQQQEEERAARPSSEQQQPVAAEAAATTTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNEL
ETMEPASAACREVITSTEGKPDPLLPITSSPENSSWDRWFQRVRSSRSNKWWQSKGSDFA

>*A.tauschiii_Gγ*3 coding sequence
ATGGCGGCGCCCAGGCCCAAGTCCCCGCTCGACCCCTGCGGCCGCCACCGGCTGCAGCTCGCCGTCGACGCGCTCCACCGCCAGATC
AGCTTCCTCGAGGGGGAGATCAGTTCCATTGAAGGGCTCCATGCTGCCTCCATATGCTGCAAAGAGGTCGATGAGTTCATAGGAAAG
AATGCCGATCCATTCATCACGATTTCATCTGAGAAGGGAAATGCCGATCAATCTCATCGCTTCCCAAAGAAGATTCGAACCCGGTGG
GCGTGTTTGAGCTGCTTCCCGTGGATCTGCGGCGGCGGGTGCTCCGCCGTCCAGCTCAAGGGGCTGAGCTGCTGCTGCGGATGCCCC
CGTTGCTGCGTGGGGAGCGGGGGCTGCGGCGGCGGACCCTCGTGTGGCTGCTCATGCTCCTGCGCCGGTTGCTCCTCCTCTTGCGCG
TGCCCTGCCTGCGCCGGCTGCGGCGCCGCGTGCTGCGGCGGTGCCCCTCGCCCTCGCTGCTGCCTGTGTTCATGA

>*A.tauschiii_Gγ*3 translation
MAAPRPKSPLDPCGRHRLQLAVDALHRQISFLEGEISSIEGLHAASICCKEVDEFIGKNADPFITISSEKGNADQSHRFPKKIRTRW
ACLSCFPWICGGGCSAVQLKGLSCCCGCPRCCVGSGGCGGGPSCGCSCSCAGCSSSCACPACAGCGAACCGGAPRPRCCLCS

>*A.tauschiii_Gγ4* coding sequence
ATGGGGGAGGGCGCGGTGGTGGTGCTGGAGGCGCCCAAGCCCAGGTCGCCGCCGAGGTACCCGGACATGTGCGGCCGCCGGCGCCTG
CAGCTGGAGGTGCAGATCCTTGACCGCGAGCTCACGTTCCTCAAGGACGAGCTACATTTACTTGAAGGGGCTCAACCAGTCTCACGT
TCTGGTTGCTTGAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGACCCGCTAATACCAATTAACAAAAGGAAGCACCGGTCCTGC
CGTCTTTACTGGTGGATCAGATCAAAACTGTGCATATGTGCTTCATGGCTGTGCTGCTCCTGCCAATGCCTACCAACTTGCAAAAGA
CCAATGTGCTTGGACTGTTCATGCTGCAAACCAAATTGCTCATGCTGCAGCCCAAACTGCTGCAGCTGCTTCAAGATCCCTTCATGC
TGCAAACCAAGCTGCGGCTGCTTCGAGTGCTGCAGCTGCAGCAAGCCACAGTGCTGCAGCAGCGGCTGTAACCCTTGCGGCGAGTGC
AAGCCCGAGTGCGGCTCATGTTCGGGCGGCGGCGGCTGCTGCGGCGGCTGCAAGCCAAGCTGCAGCTGCTGCGGCGAGCAGTGCTGC
AGCTGCGCAGGCTGCTCCTGCCCTCGGTGCACGGGGGGCTGCTTCAAGCTCCCCAAATGCTCGTGCGCGCGGTGCTTCAACTGCCAG
TCGTCGTGCTGCAAGGGGCAGCCGTCGTGCTTCAGGTGCCAGTCGTCGTGCTGCGACAAGGGAGGCTGCTGCAGCGGCGGGTCGTGC
CTGAGCTGCCCGAAGCCGTCGTGCCCCGAGTGCTCCTGCGGGTGCGTGTGGTCGTGCAAAAACTGTACAGACGGATGCCGATGCGCC
CGGTGCTGTGCTGGCGGGTGCCTGTGTTAA

>*A.tauschiii_Gγ4* translation
MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPVSRSGCLKEVNEFVGTKQDPLIPINKRKHRSC
RLYWWIRSKLCICASWLCCSCQCLPTCKRPMCLDCSCCKPNCSCCSPNCCSCFKIPSCCKPSCGCFECCSCSKPQCCSSGCNPCGEC
KPECGSCSGGGGCCGGCKPSCSCCGEQCCSCAGCSCPRCTGGCFKLPKCSCARCFNCQSSCCKGQPSCFRCQSSCCDKGGCCSGGSC
LSCPKPSCPECSCGCVWSCKNCTDGCRCARCCAGGCLC

>*H.vulgare_Gα* coding sequence
ATGGGCTCATCCTGCAGCAGACCTCACTCAGTAAACGAGGCAGAGGCAGCTGGCAACACAAGATCTGCAGACATCGACCGCCGGATT
CTGCACGAGACAAAGGCGGACCAGCACATCCACAAGCTCTTGCTTCTCGGTGCTGGAGAATCAGGAAAGTCCACGATATTTAAGCAG
ATAAAGCTTCTTTTCCGAACCGGCTTCGATGAGGCGGAACTCAAGGGCTACACGCCGGTCATCCATGCGAACGTGTACCAGACAATC
AAAATACTATATGATGGAGCTAAAGAACTTGCCCAAGTGGAACCAGAGTCCTCAAAATATGTCATATCCTCAGATAATCAGGAGATT
GGAGAAAAGCTATCAGAAATTGGAGGCAGGTTGGATTACCCACTCCTTAATAAAGAACTCGTACAGGATGTAAGAAAATTATGGGAA
GATCCAGCCATTCAGGAAACTTACTCGTGTGGAAGTGTGCTGCAAGTTCCCGACTGTGCACACTACTTCATGGAAAATCTGGACCGA
TTAGCTGAAGCAGATTATGTACCAACAAAGGAGGATGTGCTCCATGCAAGAGTACGGACAAATGGGGTTGTAGAAATTCAGTTTAGC
CCCCTTGGAGAGAGCAAAAGAGGTGGAGAGGTATACAGGCTGTATGATGTAGGAGGTCAAAGGAATGAGAGGAGGAAGTGGATTCAT
CTTTTTGAAGGCGTTGATGCTGTCATCTTTTGTGCTGCCATCAGCGAGTACGATCAGTTGTTATTTGAGGATGAGACACAGAACAGG
ATGATGGAGACCAAGGAGCTGTTCGACTGGGTATTAAAGCAAAGATGTTTTGAGAAAACATCATTCATGCTGTTCCTCAACAAGTTC
GACATATTTGAGAGGAAAATACAAAAGGTTCCTTTGACTGTGTGCGAGTGGTTTAAAGACTATGAGCCGATCGCGCCTGGCAAAGTA
CAGGATGTGGAACATGCCTATGAGTTTGTGAAAAAGAAGTTTGAGGAGGTCTACTTCCAGAGCAGCAAGCCGGATCGCGTTGACCGG
GTATTCAAGATCTACAGAACGACAGCCCTGGACCAGAAGCTTGTGAAGAAGACGTTCAAGCTGATCGACGAGAGCATGAGACGCTCC
AGGGAGGGAACGGGGACGTGA

>*H.vulgare_Gα* translation
MGSSCSRPHSVNEAEAAGNTRSADIDRRILHETKADQHIHKLLLLGAGESGKSTIFKQIKLLFRTGFDEAELKGYTPVIHANVYQTI
KILYDGAKELAQVEPESSKYVISSDNQEIGEKLSEIGGRLDYPLLNKELVQDVRKLWEDPAIQETYSCGSVLQVPDCAHYFMENLDR
LAEADYVPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAVIFCAAISEYDQLLFEDETQNR
MMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKVPLTVCEWFKDYEPIAPGKVQDVEHAYEFVKKKFEEVYFQSSKPDRVDR
VFKIYRTTALDQKLVKKTFKLIDESMRRSREGTGT

>*H.vulgare_Gβ* coding sequence
ATGGCGTCCGTGGCGGAGCTCAAGGAGAAGCACGCGGCGGCGACGGCCTCGGTGAACTCCCTCCGGGAGCGGCTCCGCCAGCGCCGG
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TGCCGCACGCTGCAGGGCCACAGCGGGAAGGTATATTCTCTGGATTGGACTCCTGAGAAGAACTGGATAGTTAGTGCCTCACAAGAT
GGACGACTAATTGTATGGAATGCTTTAACGAGTCAGAAAACACATGCCATAAAGCTACACTGTCCATGGGTGATGACATGTGCCTTT
GCACCCAATGGTCAATCTGTTGCTTGTGGCGGTCTTGATAGTGCATGCTCTATATTCAACCTTAGCTCACAAGTAGACAGAGATGGG
AACATGCCAGTATCAAGAGTACTGACTGGACACAAAGGCTATGTTTCATCATGTCAGTATGTCCCGGATCAGGAAACCCGCTTGATT
ACAGGCTCAGGTGACCAAACGTGTGTCCTGTGGGATGTTACTACTGGCCAGAGGATATCAATCTTTGGAGGCGAATTTCCATCAGGG
CATACAGCTGATGTGTTAAGTCTGTCCATCAACTCGTTAAACACGAATATGTTTGTCTCGGGTTCATGTGATACAACTGTAAGGCTA
TGGGATCTCAGGATTGCAAGCCGCGCAGTTCGGACATATCATGGACATGAGGGAGACATTAACAGCGTCAAGTTTTTCCCTGATGGT
CAGAGGTTTGGTACTGGTTCAGATGATGGTACATGCAGATTATTTGACATGAGAACAGGGCATCAACTTCAAGTGTACAATCGGGAG
CCCGACAGAAATGATAATGAACTCCCCATTGTTACATCCGTCGCCTTTTCCATATCAGGGAGGCTTCTTTTTGCTGGATACTCTAAT
GGTGACTGTTATGTGTGGGACACGCTTCTTGCCGAGGTGGTGCTTAACTTGGGAACTCTCCAAAACTCTCATGAAGGCCGTATAAGC

TGCCTTGGGCTGTCATCCGATGGGAGTGCATTGTGTACAGGAAGTTGGGACAAAAATTTGAAGATCTGGGCTTTCAGCGGACACCGC
AAGATAGTCTGA

>*H.vulgare_Gβ* translation
MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLSSQVDRDGNMPVSRVLTGHKGYVSSCQYVPDQETRLI
TGSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFVSGSCDTTVRLWDLRIASRAVRTYHGHEGDINSVKFFPDG
QRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPIVTSVAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGTLQNSHEGRIS
CLGLSSDGSALCTGSWDKNLKIWAFSGHRKIV

>*H.vulgare_Gγ*1 coding sequence
ATGCAGGCCCCGGGCGGCGTCGGTGGAGGGGAGGCGGGGGACATGCGGGGCCGGCACCGGATCCAGGCCGAGCTCAAGAAGCTCGAG
CAAGAAGCACGCTTCCTCGAGGAGGAACTTGAAAAGCTAAATAAAATGGATAAGGTGTCAGCAGCATTGCAAGAGTTTGTAGTAACA
ATTGAAAGCAAAGCAGACCCTCTACTTCCTGTAACTACCGGAGTTGCTTACCAGTCTTGGGATAGGTGGTTTGAAGGTCCGCAGGAT
CTGCGTAGATGCAAATGCTGGTTTTTGTGA

>*H.vulgare_Gγ*1 translation
MQAPGGVGGGEAGDMRGRHRIQAELKKLEQEARFLEEELEKLNKMDKVSAALQEFVVTIESKADPLLPVTTGVAYQSWDRWFEGPQD
LRRCKCWFL

>*H.vulgare_Gγ*2 coding sequence
ATGAGGGGGGAGGCCAACGGAGGGGACCGGCGGCTGCGGGACGAGGATGGGGAGGAGGAGGAACCGCCGCAGCGGCAGGAGGAGGAG
GAGAGGGCGGCGAAGCCGTCTTCTGGGCAGCAGCAGCAGCCCGCTGCCGCGGGGGCGGCGACGACCACGACGACGAGGAGCGTGGGG
TACGTGGGGAAGCACCGCCTCTCCGCCGCCATCCAGCGCCTCGACCAGGAGCTCCAGTCACTCCAGGATGAATTGAATGAGCTTGAA
ACCATGGAACCTGCATCTGCGGCATGCCGAGAGGTGATCACTAGTACTGAAGGAAAGCCTGACCCGCTTCTTCCAATCACAAGTAGC
CCGGAGAACTCTTCATGGGACAGGTGGTTCCAGCGCGTGCGAAGCTCTCGCAGCAACAAATGGTGGCAATCCAAAGGCTCCGATTTC
GCCTAG

>*H.vulgare_Gγ*2 translation
MRGEANGGDRRLRDEDGEEEEPPQRQEEEERAAKPSSGQQQQPAAAGAATTTTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELE
TMEPASAACREVITSTEGKPDPLLPITSSPENSSWDRWFQRVRSSRSNKWWQSKGSDFA

>*H.vulgare_Gγ*3 coding sequence
ATGGCGGCGCCCAGGCCCAAGTCCCCGCTCGACCCCTGCGGCCGCCACCGCCTGCAGCTCGCCGTCGACGCGCTCCACCGCCAGATC
AGCTTCCTCGAGGGGGAGATCAACTCCATTGAAGGCCTCCATGCTGCCTCCCTATGCTGCAAAGAGGTCGATGAGTTCATAGGAAAG
AATGCCGATCCACTCATAACGATTCCATCTGAGAAAGGAAACACCAATCAATCTCATCGCTCCGCAAAGAAGATCCGAGCCCGGTGG
GCGTGTTTGAGCTGCTTCCCGTGGATGTGCGGCGGCTGGTGCTCTGCAGTCCAGCGCAAGGGGCCGAGTTGCTGCTGCGGGTGTCCC
CGGTGCTGTGTGGGGAGCGGGGGCTGCGGCGGCGGACCCTCTTGTGGCTGCACATGCTCCTGCGCCGGTTGCTCCTCTTCTTGCTCG
TGCCCTGCGTGTGCCAGCTGCGGGGCCGCGTGCTGCGGGTGTGTCCCTCGCCCTCGGTGCTGCCTGTGTTCATGA

>*H.vulgare_Gγ*3 translation
MAAPRPKSPLDPCGRHRLQLAVDALHRQISFLEGEINSIEGLHAASLCCKEVDEFIGKNADPLITIPSEKGNTNQSHRSAKKIRARW
ACLSCFPWMCGGWCSAVQRKGPSCCCGCPRCCVGSGGCGGGPSCGCTCSCAGCSSSCSCPACASCGAACCGCVPRPRCCLCS

>*H.vulgare_Gγ*4 coding sequence
ATGGGGGAGGGCGCGGTGGTGGTGCTGGAGCCGCCCAAGCCCAGGTCGCCGCCGAGGTACCCGGACATGTGCGGCCGCCG
GCGCCTGCAGCTGGAGGTGCAGATCCTTGACCGCGAGCTCACGTTCCTCAAGGACGAGCTACATTTACTTGAAGGGGCTC
AACCAGTCTCACGTTCTGCTTGCTTGAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGATCCACTGATACCAATTAAC
AAAAGGAAGCATCGGTCCTGCCGTCTTTATTGGTGGATCAGATCAAAACTGTGCGTATGTGCTTCATGGCTGTGCTGCTC
CTGCCAATGCCTACCAACCTGCAAAAGACCAAGCTGCTTGGACTGTTCATGCTGCGAGCCAAACTGCTCGTGCTGCAGCC
CGAACTGCTGCAGCTGCTTCAAGATCCCTTCGTGCTGCAAACCGAGCTGCGGCTGCTTCGGGTGCTGCAGCTGCAGCAAA
CCACAGTGCTGCAGCGGCGGCTGTAACCCTTGCGGCGAGTGCAAGCCGGAGTGTGGCTCTTGTTCCGCCGGTGGCTGCTG
CGGCGACTGCAAGCCAAGCTGCAGCTGCTGCGGCGAGCAGTGCCAGTGCTGCTCCTGCCCTCGATGCACGGGGGGCTGCT
TCAAGCTCCCGAAATGCTCGTGCGCGCAGTGCTTCAACTGCCAGTCGTCGTGCTGCAAGGGGCAGCCGTCGTGCTTCAGG
TGCCAGTCGTCGTGCTGCGACAAGGGCGGCTGCTGCAGCAGCGGGTCGTGCCTGAGCTGCCCGAAGCCGTCGTGCCCGGA

GTGCTCCTGCGGGTGCGTGTGGTCGTGCAAAAACTGTACAGACGGATGCCGATGCGCCCGGTGCTGTGCTAGCGGGTGCC
TGTGTTGA

>*H.vulgare_Gγ4* translation
MGEGAVVVLEPPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPVSRSACLKEVNEFVGTKQDPLIPINKRKHRSC
RLYWWIRSKLCVCASWLCCSCQCLPTCKRPSCLDCSCCEPNCSCCSPNCCSCFKIPSCCKPSCGCFGCCSCSKPQCCSGGCNPCGEC
KPECGSCSAGGCCGDCKPSCSCCGEQCQCCSCPRCTGGCFKLPKCSCAQCFNCQSSCCKGQPSCFRCQSSCCDKGGCCSSGSCLSCP
KPSCPECSCGCVWSCKNCTDGCRCARCCASGCLC

>*B.distachyon_Gα* coding sequence
ATGGGTTCATCCTGTAGCAGACCTCACTTGAACGAGGCGGAAGCAGCTGAAAACGGAAAATCTGCAGAGATTGACCGGCGGATTTTG
CAAGAGACCAAGGCTGAGCAGCACATCCACAAGCTCTTACTTCTCGGTGCTGGAGAATCAGGAAAGTCTACGATATTTAAACAGATA
AAGCTCCTTTTCCAAACTGGCTTCGACGAGGCAGAACTGAGGAGCTATATATCAGTCATCCATGCTAACGTGTATCAGACAATTAAA
ATACTATATGATGGAGCTAAAGAACTAGCCCAAGTGGAACCAGAGTCTTCAAAGTATGTCATATCCCCAGATAATCAGGAGATTGGA
GAAAAAATATCAGAAATCGGTGGCAGGTTGGATTACCCACTGCTTTGTGAAGAACTTGTACATGACATAAGAAAATTATGGGAAGAT
CCAGCCATTCAGGAAACTTACTCACGTGGAAGTATTCTTCAAGTTCCTGACTGTGCACAGTACTTCATGGAAAATTTGGACCGATTA
GCTGAAGCAGATTATGTACCAACAAAGGAGGATGTGCTCCATGCAAGAGTACGGACAAATGGCGTTGTGGAAATTCAATTTAGCCCT
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ATATTTGAAAAGAAAATACAGAAGGTTCCTTTAACTGTGTGCGACTGGTTTAAAGACTACCAACCGATTGCGCCTGGGAAACAGGAC
GTGGAACATGCCTATGAGTTTGTCAAGAAGAAGTTTGAAGAGCTCTACTTCCAGAGTAGCAAGCCTGATCGTGTCGACCGGGTGTTC
AAGATCTACAGAACAACGGCGCTGGACCAGAAACTTGTAAAGAAGACGTTTAAGTTGATTGATGAGAGCATGAGACGCTCCAGGGAA
GAAACCTGA

>*B.distachyon_Gα* translation
MGSSCSRPHLNEAEAAENGKSAEIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIKLLFQTGFDEAELRSYISVIHANVYQTIK
ILYDGAKELAQVEPESSKYVISPDNQEIGEKISEIGGRLDYPLLCEELVHDIRKLWEDPAIQETYSRGSILQVPDCAQYFMENLDRL
AEADYVPTKEDVLHARVRTNGVVEIQFSPLGESKRGGEIYRLYDVGGQRNERRKWIHLFEGVDAVVFCAAISEYDQMLFEDEAQNRM
METKELFDWVLKQRCFEKTSFMLFLNKFDIFEKKIQKVPLTVCDWFKDYQPIAPGKQDVEHAYEFVKKKFEELYFQSSKPDRVDRVF
KIYRTTALDQKLVKKTFKLIDESMRRSREET

>*B.distachyon_Gβ* coding sequence
ATGGCGTCCGTGGCGGACCTCAAGGAGAAGCACGCGGCGGCGACGGCCTCGGTGAACTCTCTGAGAGAGAGGCTCCGTCAGCGGCGG
CAGCTGCTCCTCGACACTGACGTTGAGAGGTACTCGAAGGCGCAGGGGCGGACAGCGGTGAGCTTCAACCAGACGGATCTGGTGTGC
TGCCGCACGCTGCAGGGCCACAGCGGGAAGGTATATTCTCTTGATTGGACTCCTGAAAAGAACTGGATAGTCAGCGCCTCGCAAGAT
GGAAGATTAATTGTATGGAATGCTTTAACAAGCCAGAAAACACATGCCATAAAACTGCACTGTCCATGGGTGATGACATGTGCTTTT
GCACCCAACGGTCAATCTGTTGCCTGTGGTGGTCTTGATAGTGCATGTTCTATATTCAATCTTAACTCACAAGTAGACAGAGATGGG
AACATGCCGGTGTCAAGAATACTTACTGGACACAAAGGCTATGTTTCATCCTGTCAGTATGTCCCAGATCAGGAAACCCGGCTAATT
ACCGGTTCAGGTGACCAAACGTGTGTCCTGTGGGATGTTACTACTGGCCAAAGGATATCAATATTTGGAGGTGAATTTCCATCAGGG
CATACAGCTGATGTGCTGAGTTTGTCCATCAACCCGTTAAACACAAATATGTTTGTCTCTGGTTCATGTGATACGACTGTAAGACTT
TGGGATCTCAGAATTGCAAGCCGGGCAGTTCGAACATATCATGGACATGAGGGTGACATTAACAGTGTCAAATTTTTCCCTGATGGG
CAGAGGTTTGGTACTGGTTCAGATGATGGTACATGCAGACTATTTGACATGAGAACAGGGCATCAACTTCAAGTGTACAACAGGGAG
CCAGATAGAAATGATAATGAGCTCCCTATTGTTACTTCAATTGCATTTTCTATATCCGGGAGGCTTCTTTTTGCTGGATACTCTAAT
GGTGACTGTTACGTGTGGGATACACTTCTTGCTGAGGTGGTGCTTAATTTGGGAACCCTTCAGAACTCTCATGATGGCCGTATAAGC
TGCCTTGGGCTGTCATCTGATGGGAGCGCATTGTGTACAGGAAGTTGGGACAAAAATTTGAAGATTTGGGCTTTCAGTGGACACCGC
AAAATAGTTTGA

>*B.distachyon_Gβ* translation
MASVADLKEKHAAATASVNSLRERLRQRRQLLLDTDVERYSKAQGRTAVSFNQTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLNSQVDRDGNMPVSRILTGHKGYVSSCQYVPDQETRLI
TGSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINPLNTNMFVSGSCDTTVRLWDLRIASRAVRTYHGHEGDINSVKFFPDG

QRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPIVTSIAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGTLQNSHDGRIS
CLGLSSDGSALCTGSWDKNLKIWAFSGHRKIV

>*B.distachyon_Gγ*1 coding sequence
ATGCAGGTTCCAGGCGGCGGCGGCGGAGGAGGAGCTGGAAGGGAAGCGGGGGACACGCGGGGCCGGCACCGGATCCAGGCTGAGCTC
AAGAAGCTGGAGCAAGAAGCGCGCTTCCTCAAGGAGGAACTTCAAGAGCTAGAGAAAACGGATATAATATCAGCAGCATTGCAAGAG
TTTCTTGTAACAATTGAAGGAAAAGCAGACCCTCTACTTCCTGTAACTACCGGAGTGGCTTACCAGTCTTGGGATAGGTGGTTTGAA
GGTCCAGAAGATCTGCGTAGATGCAAATGCTGGTGTCTGTGA

>*B.distachyon_Gγ*1 translation
MQVPGGGGGGGAGREAGDTRGRHRIQAELKKLEQEARFLKEELQELEKTDIISAALQEFLVTIEGKADPLLPVTTGVAYQSWDRWFE
GPEDLRRCKCWCL

>*B.distachyon_Gγ*2 coding sequence
ATGAGAGGAGAAGCCAACGGAGAGGGCCGCGGGGAGGAAGAGCAGCAGCAGCAGCAGGTGCAGGAGGGGGAGGAGGCGGATGGCGCG
GCGAGGCCGTCTTCAGGGCAGCAGCAGCCAGCGGTGGCTGCGGCGGCGACGACGAGGGGCGTGGGGTACGTCGGGAAGCACCGCCTC
TCCGCCGCCATCGCCCGCCTCGACCAGGAGCTGCAGTCTCTCCAGGATGAATTGAATGAGCTTGAAACCATGGAACCTGCATCCGCA
GCATGCCAGGAGGTGATCACAAGTACCCAAGGAAAACCTGACCCACTTCTTCCAATCACCAGTAGCCCCGAGAACTCTTCCTGGGAC
AGATGGTTCCAGCGTGTGCCAAGCTCTCGCAGCAGCAAGTGGTGGACCTCCAAAGGCTCCAATTTTTCCTAG

>*B.distachyon_Gγ*2 translation
MRGEANGEGRGEEEQQQQQVQEGEEADGAARPSSGQQQPAVAAAATTRGVGYVGKHRLSAAIARLDQELQSLQDELNELETMEPASA
ACQEVITSTQGKPDPLLPITSSPENSSWDRWFQRVPSSRSSKWWTSKGSNFS

>*B.distachyon_Gγ*3 coding sequence
ATGATGGCAATGGTGGCGCCCAGGCCCAAGTCGCCGCCGGCCTCGCCGGACCCCTGCGGCCGACACCACCTGCAGCTCGCCGTCGAC
ACGCTCCACCGCGAGATCGGATTCCTCGAGGGGGAAATTAGTTCCGTTGAAGGGGTGCATGCTGCCTCCAAATGCTGCAAAGAGGTT
GATGAGTTCGTAGGAAAGAATGCCGATCCATTCATAACGATTTCATCAAAGAAGGCGAACACCGATCAGTCTCGCCACCTTCCAAAG
AAGTTTCGAGCCCGGACGTGTTTGAGCTACCTGTCGTGGATGTGCTGCTGCGGCGGGTGTCCTTCCGTCCAGCTCCAGGGGCCAACG
AGCTGCTGCTCTTGCGGAGCGCTGGGAGGGCTGTGCGGTTGCTGCAGCACCGGAGAATGCTGCCGCTGCCGCGTCGGGTGCGGGGGC
TGCGGCTGCTGCTGCTGCTGCTGCCGTGGCAGCCCGTGCCGCAGCCGCACGCCGAGCCCGAGATGCTCGTGTGGTTGCACGTGCTCC
TGCCCGAGCTGCTGCTCCTCCTCTTGCGCGTGTCCGGCTCCCTCCTGCTGCCGTGCCCCTCGGTGTTGTTACCTGTGTTC
ATGA

>*B.distachyon_Gγ*3 translation
MMAMVAPRPKSPPASPDPCGRHHLQLAVDTLHREIGFLEGEISSVEGVHAASKCCKEVDEFVGKNADPFITISSKKANTDQSRHLPK
KFRARTCLSYLSWMCCCGGCPSVQLQGPTSCCSCGALGGLCGCCSTGECCRCRVGCGGCGCCCCCCRGSPCRSRTPSPRCSCGCTCS
CPSCCSSSCACPAPSCCRAPRCCYLCS

>*B.distachyon_Gγ*4 coding sequence
ATGGGGGAGGCGCCGAGGCCCAAGTCGCCGCCCAAGTACCCGGACCTGTGCGGCCGCCGGCGCCTGCAGCTGGAGGTGCAGAGCCTC
AACCGGGAGGTCGGCTTCCTCGAGCAAGAGCTACAGGGACTTGAACGGATGCAGCCGGTCTCGAGGTGTTGCAAAGATGTCAACGAA
TATGTCGGTGCAAAGACGGACCCACTTATACCAATAAACAAAAGGAAACACAGATCTTGCAGTCTCTACCGGTGGATCAGATCGAAG
CTGTGCACCTGCTTTTCGTGCCTGTGCTGCTGGTGCCGATGCCTGCCCAAGAGACCAAGCTGCTTCACCTGTTCGTGCTGCTCCTGC
GGCGACACGTCGTGCTGTACACCGAGCTGCAGCTGCCTGAACAAGACCCCTTCATGCTGCAAACCCCAATGCGGCGGCGGCAGCTCC
GATTGCTGCAGCCTTCCCAGCTGCTGCGACTGCAAGACACATTGCACAGGCTGCGGCGATTGCCATTGCCAGCCGCAGTGCTGCTGC
AAGCCGAGCTGCTCGTGCTCTTTGCCCAGCTGCTGCTGTAGCCTCTCCTCCGGCAGCAGCTGCGGCTGCGCCGAGAAGTGCTCCTGC
ACGCCGTGCTTGGGCTGCCTGGGAGTCTTCTTCGAGCGGTGCCTCAGCTGCCGGTCGTCGTGCTGCAAGGGGCAGCAGCCGTCCTGC
TGCAAGTGCCAGCTTTCGTGTTGCGAGGGAGATGAGTCGTCGTCGTGCTGCGGCAGAGGGGCGTGTGACTCGTGCAAGTCGTGCTTC
GGCGCGCCGTCGTGCCCGGAGTGCTCCTGCGGCTGCGTGTGCTCCTGCCCGAGGTGCAAGGGGGGGTGCCGGTGCCCGTCGTGCGGC
AACCCGTGCGGTGCCGGGGGATGCTTGTGCTAG

>*B.distachyon_Gγ*4 translation
MGEAPRPKSPPKYPDLCGRRRLQLEVQSLNREVGFLEQELQGLERMQPVSRCCKDVNEYVGAKTDPLIPINKRKHRSCSLYRWIRSK
LCTCFSCLCCWCRCLPKRPSCFTCSCCSCGDTSCCTPSCSCLNKTPSCCKPQCGGGSSDCCSLPSCCDCKTHCTGCGDCHCQPQCCC
KPSCSCSLPSCCCSLSSGSSCGCAEKCSCTPCLGCLGVFFERCLSCRSSCCKGQQPSCCKCQLSCCEGDESSSCCGRGACDSCKSCF
GAPSCPECSCGCVCSCPRCKGGCRCPSCGNPCGAGGCLC

>*S.cereale_Gα* coding sequence

ATGGGCTCCTCCTGCAGCAGACCTCACTCCGTAAACGAGGCCGAGGCAGCCGACAACAGAAGATCTGCAGACATCGACCGGCGGATT
CTGCAGGAGACAAAGGCGGATCAGCACGTCCACAAGCTCTTGCTTCTCGGTGCTGGAGAATCAGGAAAGTCCACGATATTTAAGCAG
ATTAAGCTTCTTTTTCGAACCGGCTTCGACGAGGCAGAACTCAAGGGCTATACGCCGGTCATCCATGCCAACGTGTTCCAGACAATC
AAAATACTGTATGATGGAGCTAAAGAGCTTGCCCAAATGGAAACTGAGTCTTCAAAACATGTTATATCCCCGGATAACCAGGAGATT
GGAGAAAAACTATCAGAAATCGGAGGCAGGTTGGATTACCCACTCCTTAACAAAGAACTCGTACAGGATGTAAGAAAATTATGGGAA
GATCCAGCCATTCAGGAAACTTACTCGTGTGGAAGTGTGCTGCAAGTTCCTGACTGTGCACACTACTTTATGGAGAATCTGGACCGA
TTAGCTGAACCAGATTATGTACCAACAAAGGAGGATGTGCTCCATGCCAGAGTACGGACAAATGGGGTTGTGGAAATTCAATTTAGC
CCCGGAGAGAGCAAAAGAGGCGGAGAGGTATACAGGTTGTACGATGTAGGAGGTCAAAGGAATGAGAGGAGGAAGTGGATTCATCTT
TTTGAAGGCGTCGATGCCGTCATCTTTTGCGCTGCCATTAGCGAGTATGATCAGCTGTTGTTTGAGGACGAGACACAGAACAGAATG
ATGGAGACGAAGGAACTGTTCGACTGGGTACTAAAGCAAAGATGTTTTGAGAAAACATCGTTCATGCTGTTCCTCAACAAATTTGAC
ATATTTGAGAGGAAAATACAAAAGGTTCCTTTGACCGTGTGCGAGTGGTTTAAAGACTATGAGCCGATCGCGCCTGGCAAACAGGAT
GTGGAACATGCCTATGAGTTTGTGAAGAAGTTTCAGGTCTACTTCCAGAGCAGCAAGCCAGACCTCGTCGACCGGGTGTTCAAGATC
TACAGAACGCCGCGGGAGGACCAGAAACTTGTCATGAAGACGTTCAAGCTGATCGACGAGAGCATGAGAGGCTCCAGGGAGGGAACT
GGGTAG

>*S.cereale_Gα* translation

MGSSCSRPHSVNEAEAADNRRSADIDRRILQETKADQHVHKLLLLGAGESGKSTIFKQIKLLFRTGFDEAELKGYTPVIHANVFQTI
KILYDGAKELAQMETESSKHVISPDNQEIGEKLSEIGGRLDYPLLNKELVQDVRKLWEDPAIQETYSCGSVLQVPDCAHYFMENLDR
LAEPDYVPTKEDVLHARVRTNGVVEIQFSPGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVDAVIFCAAISEYDQLLFEDETQNRM
METKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKVPLTVCEWFKDYEPIAPGKQDVEHAYEFVKKFQVYFQSSKPDLVDRVFKI
YRTPREDQKLVMKTFKLIDESMRGSREGTG

>*S.cereale_Gβ* coding sequence

ATGGCGTCCGTGGCGGAGCTCAAGGAGAAGCACGCGGCGGCGACGGCCTCGGTCAACTCCCTGCGGGAGCGGCTCCGCCAGCGCCGG
CAGACGCTCCTCGACACCGACGTGGAGAAGTACTCCAAGGCGCAGGGGCGGACGGCGGTGAGCTTCAACCCCACGGATCTGGTGTGC
TGCCGCACGCTGCAGGGCCACAGCGGAAAGGTATATTCTTTGGATTGGACTCCTGAGAAGAACTGGATAGTTAGTGCCTCCCAAGAT
GGAAGACTAATTGTATGGAATGCTTTAACGAGTCAGAAAACACATGCCATAAAGCTACACTGTCCATGGGTGATGACATGTGCTTTT
GCACCCAATGGTCAATCTGTTGCTTGTGGCGGTCTTGATAGTGCATGCTCTATATTCAACCTTAGCACACAAGCAGACAGAGATGGG
AACATGCCAGCATCAAGAGTACTTACTGGACACAAAGGCTATGTTTCATCCTGTCAGTATGTCCCGGATCAGGAAACCCGCTTGATT
ACAGGCTCGGGTGACCAAACTTGTGTCCTGTGGGATGTTACTACTGGCCAGAGGATATCAATCTTTGGAGGCGAATTTCCATCAGGG
CATACAGCTGATGTGTTAAGTCTGTCCATCAACTCGTTAAACACGAATATGTTTATCTCGGGTTCATGTGATACAACTGTAAGGCTA
TGGGATCTCAGGATTGCAAGCCGGGCAGTTCGGACATATCATGGACATGAGGGCGACATTAACAGTGTCAAGTTTTTCCCTGATGGT
CAGAGGTTCGGTACTGGTTCAGATGATGGTACATGCAGATTATTTGACATGAGAACGGGGCATCAACTTCAAGTGTACAATCGGGAG
CCCGATAGAAATGATAATGAGCTCCCTATTGTTACATCTGTCGCCTTTTCCATATCAGGAAGGCTCCTTTTTGCTGGATACTCTAAT
GGTGACTGTTATGTGTGGGACACGCTTCTTGCCGAGGTGGTGCTCAATTTGGGAACTCTCCAAAACTCCCATGAAGGCCGTATAAGC
TGCCTTGGGCTGTCATCCGATGGGAGTGCACTGTGTACAGGAAGTTGGGACAAGAATTTGAAGATCTGGGCTTTCAGCGGACACCGC
AAGATAGTCTGA

>*S.cereale_Gβ* translation

MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLSTQADRDGNMPASRVLTGHKGYVSSCQYVPDQETRLI
TGSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFISGSCDTTVRLWDLRIASRAVRTYHGHEGDINSVKFFPDG
QRFGTGSDDGTCRLFDMRTGHQLQVYNREPDRNDNELPIVTSVAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGTLQNSHEGRIS
CLGLSSDGSALCTGSWDKNLKIWAFSGHRKIV

>*S.cereale__Gγ*1 coding sequence

ATGCAGGTTCCGGGCGACGTCCGCGCCGGTGGAGGGGAAGCGGGGGACATGCGGGGCCGGCACCGGATCCAGGCCGAGCTCAAGAAG
CTCGAGCAAGAAACACGCTTCCTCGAGGAGGAACTTGAAGAGCTAGATAAAATGGACAAGGTATCAACAGCATTGCAAGAGTTTGTG
GTAACAATTGAAAGCAAAGCAGACCCTCTACTTCCTGTAACTACCGGAGCTGCTTACCAGTCTTGGGATAGGTGGTTTGAAGGTCCG
CAGGATCTGCGTAGATGCAAATGCTGGTTCTTGTGA

>*S.cereale__Gγ*1 translation

MQVPGDVRAGGGEAGDMRGRHRIQAELKKLEQETRFLEEELEELDKMDKVSTALQEFVVTIESKADPLLPVTTGAAYQSWDRWFEGP
QDLRRCKCWFL

>*S.cereale_Gγ*2 coding sequence

ATGAGGGGGGAGGCCAACGGAGGGGACCGGCGGCCGCGGGACGAGGAGGAGGAGGAGGAGGAGCCGCCGCCGCCGCAGCAGCAGCAGGAG
GAGAGGGCGGCGAGGCCGTCTTCTGGGCAGGAGCAGCAGCAGCCGGCTGCGGCGGCGGCGGCGGCGACGACGACGAGGAGCGTGGGG

TACGTGGGGAAGCACCGCCTCTCCGCCGCCATCCAGCGCCTCGACCAGGAGCTCCAGTCACTCCAGGATGAATTGAATGAGCTTGAA
ACCATGGAACCTGCATCTGCGGCGTGCCGCGAGGTGATCACAAGTACTGAAGGAAAACCTGACCCGCTTCTTCCAATCACAAGTAGC
CCGGAGAACTCTTCATGGGACAGGTGGTTCCAGCGCGTGCGAAGCTCTCGCAGCAACAAATGGTGGCAATCCAAAGGCTCCGATTTT
GCCTAG

>*S.cereale__Gγ2* translation
MRGEANGGDRRPRDEEEEEEEPPPQQQQEERAARPSSGQEQQQPAAAAAAATTTRSVGYVGKHRLSAAIQRLDQELQSLQDELNELE
TMEPASAACREVITSTEGKPDPLLPITSSPENSSWDRWFQRVRSSRSNKWWQSKGSDFA

>*S.cereale_Gγ3* coding sequence
ATGGGGGAGGGCGCGGTGGTGGTGCTGGAGGCGCCCAAGCCCAGGTCGCCGCCGAGGTACCCGGACATGTGCGGCCGCCGGCGCCTG
CAGCTCGAGGTGCAGATCCTTGACCGCGAGCTCACGTTCCTCAAGGACGAGCTACATTTACTTGAAGGGGCTCAGCCAGTCTCCCGT
TCTGGTTGCTTGAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGACCCACTAATACCAATTAACAAAACAAAGCACCGGTCCTGC
CGTCTTTATTGGTGGATCAGATCAAAACTGTGCATATGTGCTTCATGGCTGTGCTGCTCCTGCCAATGCCTACCAACCTGCAAAAGA
CCAAGGTGCTTCGACTGTTCATGCTGCGAGCCAAACTGCTCATGCTGCAGTCCGAACTGCTGCAGCTGCTTCAAGATCCCTTCATGC
TGCAAACCAAGCTGCGGCTGCTTCGACTGCTGCAGCTGCAGCAAACCGCAGTGCTGCAGCAGCGGCTGTAACCCTTGCGGCGAGTGC
AAGCCAGAGTGCGGCTCATGTTCCGGTGGCGGCTGCTGCGGTGACTGCAAGCCAAGCTGCAGCTGCTGCGGCGAGCAGTGCTGCAGC
TGCGGGGGCTGCTCCTGCCCTAGATGCGCGGGTGGCTGCTTCAAGCTCCCCAAGTGCTCGTGCGCGCAGTGCTTCAACTGCCAGTCG
TCGTGCTGCAAGGGGCAGCCGTCGTGCTTCAGGTGCCAGTCGTCGTGCTGCGACAAGGGAGGCTGCTGCAGCGGCGGGTCGTGCGTG
AGCTGCCCGAAGCCGTCGTGCCCCGAGTGCTCCTGCGGGTGCGTGTGGTCGTGCAAAAACTGTACAGACGGATGCCGATGCGCCCGG
TGCTGTGCTGGCGGGTGCCTGTGTTAA

>*S.cereale_Gγ3* translation
MGEGAVVVLEAPKPRSPPRYPDMCGRRRLQLEVQILDRELTFLKDELHLLEGAQPVSRSGCLKEVNEFVGTKQDPLIPINKTKHRSC
RLYWWIRSKLCICASWLCCSCQCLPTCKRPRCFDCSCCEPNCSCCSPNCCSCFKIPSCCKPSCGCFDCCSCSKPQCCSSGCNPCGEC
KPECGSCSGGGCCGDCKPSCSCCGEQCCSCGGCSCPRCAGGCFKLPKCSCAQCFNCQSSCCKGQPSCFRCQSSCCDKGGCCSGGSCV
SCPKPSCPECSCGCVWSCKNCTDGCRCARCCAGGCLC

>*S.italica_Gα* coding sequence
ATGGGCTCATCCTGTAGCAGACATCATTCCTTAAACGAGGCTGAAGCAGCTGAAAATGCAAAGTCTGCTGACATCGACCGGCGGATT
TTGCAAGAGACAAAGGCAGAGCAACACATCCACAAGCTCTTACTTCTCGGTGCTGGAGAATCGGGAAAGTCTACAATATTTAAACAG
ATAAAGCTCCTTTTCCAAACTGGTTTCGATGAGGCAGAACTTAGAAGCTATACTTCAGTCATCCATGCGAACGTATATCAGACAATT
AAAATTCTATATGACGGAGCTAAAGAGCTAGCCCAAGTGGAACCAGATTCCTCAAAATATGTTCTATCCCCAGATAATCAGGAGATT
GGGGAGAAACTATCAGAAATTGGTGCAAAATTGGATTACCCATTGCTGAACAAAGAACTTGTACAGGATGTAAGGAAATTATGGCAA
GATCCAGCCATTCAGGAAACTTATTCACGCGGAAGTATTCTGCAAGTCCCTGACTGTGCACAGTACTTCATGAGCAATTTGGACCGA
TTAGCTGAAGTAGATTATGTACCAACAAAGGAGGACGTGCTTCATGCAAGAGTACGGACGAATGGTGTGGTAGAAACTCAGTTTAGC
CCTCTTGGAGAGAGCAAAAGAGGTGGAGAGGTCTATAGGTTGTACGATGTAGGAGGCCAAAGAAATGAGAGAAGGAAGTGGATTCAT
CTTTTTGAAGGTGTTAATGCTGTAATCTTTTGTGCTGCCGTTAGCGAGTACGATCAGATGTTATTTGAGGATGAGACGAAGAACAGA
ATGATGGAGACCAAAGAACTCTTTGACTGGGTCCTAAAGCAAAGATGTTTTGAGAAAACCTCATTCATGTTGTTTCTCAACAAATTC
GACATATTTGAGAGGAAAATACAAAAGGTTCCTTTGAGTGTGTGTGAGTGGTTTAAAGACTACCAGCCTACTGCGCCTGGCAAACAG
GAGGTTGAACATGCCTACGAGTTTGTGAAAAAGAAGTTTGAGGAGCTCTACTTCCAGAGCAGCAAGCCAGACCGC
GTAGACCGAGTGTTCAAGATTTACAGAACAACCGCCCTGGATCAGAAACTTGTGAAGAAGACATTTAAGTTGATCGACGAGAGCATG
AGGCGCTCCAGAGAAGGAACCTGA

>*S.italica_Gα* translation
MGSSCSRHHSLNEAEAAENAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIKLLFQTGFDEAELRSYTSVIHANVYQTI
KILYDGAKELAQVEPDSSKYVLSPDNQEIGEKLSEIGAKLDYPLLNKELVQDVRKLWQDPAIQETYSRGSILQVPDCAQYFMSNLDR
LAEVDYVPTKEDVLHARVRTNGVVETQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAVIFCAAVSEYDQMLFEDETKNR
MMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKVPLSVCEWFKDYQPTAPGKQEVEHAYEFVKKKFEELYFQSSKPDRVDRV
FKIYRTTALDQKLVKKTFKLIDESMRRSREGT

>*S.italica_Gβ* coding sequence
ATGGCGTCCGTGGCGGAGCTCAAGGAGAAGCACGCCGCGGCGACGGCGTCGGTCAACTCCCTCCGCGAGCGCCTTCGCCAGCGCCGG
GAGATGCTCCTCGACACCGACGTGGCGAGGTACTCCAAGGCGCAGGGGAGGACGCCGGTGAGCTTCAACCCGACGGATCTGGTCTGC

TGCCGCACGCTGCAGGGCCACAGCGGAAAGGTATATTCTCTGGATTGGACTCCCGAAAAGAATTGGATAGTCAGTGCCTCACAAGAT
GGAAGGCTGATTGTGTGGAATGCGTTAACAAGCCAGAAAACACATGCCATAAAGCTGCACTGCCCATGGGTGATGACATGCGCTTTT
GCACCCAATGGCCAGTCTGTTGCCTGTGGTGGTCTAGATAGTGCATGCTCTATTTTCAATCTTAACTCGCAAGCAGACAGAGACGGG
AATATGCCAGTATCAAGAATTCTTACTGGACACAAGGGCTATGTTTCGTCATGCCAATATGTCCCAGATCAGGAAAGTCGCCTTATT
ACAAGCTCAGGTGATCAGACATGTGTTCTGTGGGATGTTACTACTGGCCAGAGGATATCAATATTTGGAGGTGAATTTCCATCAGGG
CATACAGCTGATGTTCAAAGTGTATCCATCAACTCATCGAATACGAATATGTTTGTCTCTGGCTCATGTGATGCAACTGTGAGGCTG
TGGGATATCAGAATTGCAAGTCGGGCTGTTCGAACCTATCATGGACATGAGGCTGACGTTAACAGTGTGAAGTTTTTCCCTGATGGC
CATAGGTTTGGTACTGGCTCAGATGATGGTACTTGTAGATTATTTGACATGAGAACTGGGCATCAACTTCAGGTGTACAGTAGGGAG
CCTAATAGAGATGATAATGAACTACCTACTGTTACATCTATCGCATTCTCGATATCAGGAAGGCTACTATTTGCTGGGTACTCCAAT
GGTGACTGTTACGTGTGGGACACACTTCTTGCCGAGGTGGTACTTAATTTGGGAAACCTCCAAAACTCCCATGATGGTCGTATAAGT
TGCCTTGGAATGTCATCTGATGGGAGTGCATTGTGTACAGGAAGTTGGGACAAAAATTTGAAGATTTGGGCCTTCAGTGGACACCGG
AAAATAGTTTGA

>*S.italica_Gβ* translation
MASVAELKEKHAAATASVNSLRERLRQRREMLLDTDVARYSKAQGRTPVSFNPTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLNSQADRDGNMPVSRILTGHKGYVSSCQYVPDQESRLI
TSSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDATVRLWDIRIASRAVRTYHGHEADVNSVKFFPDG
HRFGTGSDDGTCRLFDMRTGHQLQVYSREPNRDDNELPTVTSIAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGNLQNSHDGRIS
CLGMSSDGSALCTGSWDKNLKIWAFSGHRKIV

>*S.italica_Gγ*1 coding sequence
ATGCAGGT
CGGCGGCGGCGGCGCCGGAGGAGGGGATGCGGCAGACATACGGGGCCGCCACCGGATCCAGGCCGAGCTCAAGAAGCTCG
AGCAAGAAGCGCGCTTCCTCGAGGAGGAACTTGAAGAGCTTCAGAAAACTGATAAGGTATCATCGGCATTGCAAGAGTTT
CTAACAGCGATGGAAAGCAAAGCAGATCCTCTACTTCCTGTAACTACTGGACCTGTGAATCAGTCCTGGGATAGGTGGTT
TGAAGGTCCACAAGATCTGCGCAGATGCAAATGCTGGTTTTTGTGA

>*S.italica_Gγ*1 coding sequence
MQVGGGGAGGGDAADIRGRHRIQAELKKLEQEARFLEEELEELQKTDKVSSALQEFLTAMESKADPLLPVTTGPVNQSWDRWFEGPQ
DLRRCKCWFL

>*S.italica_Gγ*2 coding sequence
ATGAGGGGGGAGGCCAACGGAGGGGAGGACCGCCGGCCGCGGGGCGAGGACCAGGAGCACGAGGACGACGAGGAGGAGGAGCGGCGC
GGGGGCGAGGGAGCGCCGCCTCAGAGGCACGTGCAGGCGCAGAGGCCCGCCGCGCGGCCTTCCACCGACCCGCAGCACCAGCAGCAC
CCGCCGCCGCCGCCGGGGGTGATGAGGAACGTCGGCTACGTCGGCAAGCACCGCCTCTCCGCCGCCATCAGCCGCCTCGACCAGGAG
CTCCAGTCCCTCCAGGAGGAACTGAATGAGCTTGAAACCATGGAACCTGCATCTACTGCATGCCAGGATGTGATCACAAGTACAGAA
GGGAAACCAGACCCGCTTCTTCCTATCACCAGTGGTCCGGAGAACTCTTCTTGGGACAGATGGTTTCAGCGGGTTCGCAGCTCCCGC
AGCAACAAATGGTGGGCATCAAGAGGCTCTGACTTCTCTTAG

>*S.italica_Gγ*2 translation
MRGEANGGEDRRPRGEDQEHEDDEEEERRGGEGAPPQRHVQAQRPAARPSTDPQHQQHPPPPPGVMRNVGYVGKHRLSAAISRLDQE
LQSLQEELNELETMEPASTACQDVITSTEGKPDPLLPITSGPENSSWDRWFQRVRSSRSNKWWASRGSDFS

>*S.italica_Gγ*3 coding sequence
ATGGCTGCTGCGCCGGCGGCACCCAGGCCCAAGTCGCCGCCGGCCTCGCCCGACCCGTGCGGCCGCCACCGCCTGCAGCTCGCCGTC
GACGCGCTCCACCGGGAGATCGGCTTCCTCGAGGGTGAAATAAGTTCCATTGACGGGGTCCACGCTGCCTCCAGATGCTGCAAAGAG
GTTGATGAGTTTGTGGGAAGAAATCCCGATCCATTCATAACGATTCAGCCAGAGAAACGAAGCAATGAGCAGTCTCAGCAGTTTCTG
AAGAAGTTCCGAGCCAAGAGCTGCCTGAGCTACCTGTCGTGGATCTGCTGCGGCGGCGGCGGGTGCCCGCCGTTCCAGCTGAAGACG
ACGATGAGGCCGCCGTCGGCGAGCTGCTCCTGCGGCGGCGCGCGGCTGCGGAAGCTCTGCTCGTCCCCGTGCTGCTGCTGCTGCTGC
TGCAGGTGCCGCGTGGTGTACGCCGGGTGCTGCGCGCCGTGCCCGCGCTGCTCGTGCGGCTGCGCCTGCCCGCGGTGCTCGTCGTGC
GCCTGCTGCCCCACCTGCAGCGACGCGTGCTGCGCCCCGCGCTGCTGCCTGTGCCTATGA

>*S.italica_Gγ*3 translation
MAAAPAAPRPKSPPASPDPCGRHRLQLAVDALHREIGFLEGEISSIDGVHAASRCCKEVDEFVGRNPDPFITIQPEKRSNEQSQQFL
KKFRAKSCLSYLSWICCGGGGCPPFQLKTTMRPPSASCSCGGARLRKLCSSPCCCCCCCRCRVVYAGCCAPCPRCSCGCACPRCSSC
ACCPTCSDACCAPRCCLCL

>*S.italica_Gγ4* coding sequence

ATGGGGGAGCAGGTGGCTGTGGTGCTGGAGCCGCCGCGGCCCAAGTCGCCGCCGAGGTACCCGGACTTGTGCGGCCGCCGCCGGCTG
CAGCTGGAGCTCCAGATCCTCAACCGCGAGGTCGACTTCCTCAAGGACGAGCTACAATCACTTGAAGGAGTTCCACCAGTTTCTAGA
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AAGTGCCAGTCGTCTTGCTGCGAGGGTGAAGACGGCAGCAGCTGCTGCTGGAGATCGTGCTGCAGCGTTCCGAAGCCTTCGTGCCCC
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GGATGCTTGTGCTGA

>*S.italica_Gγ4* translation

MGEQVAVVLEPPRPKSPPRYPDLCGRRRLQLELQILNREVDFLKDELQSLEGVPPVSRSCKEVNEFVGTKQDPLLPIEKKRHRSCGL
FWWIGSKLCICVPWICCSCQCLPKCKRPCCFDCSCCSCPDVSCCKPSCKSCNKPCCGPNSCSCCNVSCCKPDCPSCSPSCSSCCKPN
CSSCCSPSCCKPNCSTCCKPNCGSCCKPKCSCFGLPSCCKFQCSPNCCTCTMPSCSGCNPCGSCKGCCSCPSDCCNRKPNCSCFSAQ
CCSCAECYSCTCPSCSSCFSCFGCFKSWKCSNLFGGCCSCKQCFKCQSSCCKGAPSCCKCQSSCCEGEDGSSCCWRSCCSVPKPSCP
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>*S.bicolor_Gα* coding sequence

ATGGGTTCATCCTGTAGCAGATCCCATTCCTTGGACGAGACTGAAGCAGCTGAAAATGCAAAGTCTGCAGACATCGACCGGAGGATT
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CCTCTAGGAGAGAGCAAAAGAGGCGGAGAGGTCTATAGGCTATACGATGTAGGAGGCCAGAGAAACGAGAGAAGGAAATGGATCCAT
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GAAGGAACTTGA

>*S.bicolor_Gα* translation

MGSSCSRSHSLDETEAAENAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIKLLFQTGFDEAELKSYTSVIHANMYQTI
KILYEGAKELAQVEPDSSKYVLSPDSQEIGEKLSEIGVRLDYPSLNKERVQDVRKLWQDPAIQETYSRGSILQVPDCAQYFMENLDR
LSEVDYVPTKEDVLHARVRTNGVVETQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAVIFCAAISEYDQMLCEDETKNR
MMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKVPLSACEWFKDYQPIAPGKQEVEHAYEFVKKKFEELYFQSSKPDRVDRV
FKIYRTTALDQKLVKKTFKLIDESMRRSREGT

>*S.bicolor_Gβ* coding sequence

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GAGACGCTCCTCGACACGGACGTGGCGAGGTACTCCAAGTCGCAGGGGAGGCTGCCGGTGAGCTTCAACCCGACGGATCTGGTCTGC
TGCCGCACGCTGCAGGGCCATAGCGGAAAGGTATATTCTCTGGATTGGACCCCTGAAAAGAATTGGATAGTCAGTGCCTCTCAAGAT
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GTCGTATAAGTTGCCTTGGGATGTCATCTGATGGGAGTGCATTGTGTACAGGGAGTTGGGACAAAAATTTGAAGATTTGGGCCTTCA
GTGGACACCGGAAAATAGTTTGA

>*S.bicolor_Gβ* translation
MASVAELKEKHAAATASVNSLRERLRQRRETLLDTDVARYSKSQGRLPVSFNPTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLNSQADRDGNMPVSRILTGHKGYVSSCQYVPDQETRLI
TSSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDTTVRLWDIRIASRAVRTYHGHEGDVNSVKFFPDG
HRFGTGSDDGTCRLFDMRTGHQLQVYSRVPDRNDDELPTVTSIAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGNLQNSHDGRIS
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>*S.bicolor_Gγ*1 coding sequence
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CTCGAGCAGGAGGCGCGCTTCCTCGAGGAAGAACTTGAAGAGCTTGAGAAAGCAGATAAGGTATCATCTGCACTGCAAGAGCTTCTA
ACGGCAATGGAAAGGAAAGCTGACCCTCTACTTCCTGTATCTACTGGACCTGTGAATCAGTCCTGGGATAGGTGGTTTGAAGGTCCA
CAAGATCTGCGCAGATGCAAATGCTGGTTTTTGTGA

>*S.bicolor_Gγ*1 translation
MQVGGGGGGGGGDSADLRGRHRIQAELKKLEQEARFLEEELEELEKADKVSSALQELLTAMERKADPLLPVSTGPVNQSWDRWFEGP
QDLRRCKCWFL

>*S.bicolor_Gγ*2 coding sequence
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CAGACACAGAGACCTTCCTCCGGCCCGCAGCAGCAGCAGCAGCACCAGCCGCCGCCGCTCACCAGGAACGTCGGCTACGTCGGC
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TCTGCATCCGCGGCATGCCAGGAAGTGATCACGAGTACACAAGGGAAACCTGACCCTCTTCTTCCTATTACCAGTGGTCCAGAGAAC
TCTTCATGGGACAGATGGTTTCAGCGGGTCCGCTCCCGCAGCAACAAATGGTGGGCATCAAAAGGCTCTGACTTCTCGTAG

>*S.bicolor_Gγ*2 translation
MRGQANGVEDRRPRGDDHEADDDEEDSEEEEEEEGRHRGQGQGQGQGQGPPPQQRRHQQTQRPSSGPQQQQQQHQPPPLTRNVGYVG
KHRLSAAIARLDQELQSLQEELDELETMESASAACQEVITSTQGKPDPLLPITSGPENSSWDRWFQRVRSRSNKWWASKGSDFS

>*S.bicolor_Gγ*3 coding sequence
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CACCGGGAGATCAGCTTCCTCGAGGGCGAAATAAGTTCCATTGAGGGGGTCCACGCTGCCTCCAGATGCTGCAAAGAGGTTGATGAG
TTTGTTGGAAGTAATCCGGATCCATTCCTAACGATTCAGCCAGAGAAAGGAAGCCATGATCAATCTCAGCAGTTTCTGAAGAAGTTC
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>*S.bicolor_Gγ*3 translation
MAAAPRPKSPPASPDPCGRHRLQLAVDALHREISFLEGEISSIEGVHAASRCCKEVDEFVGSNPDPFLTIQPEKGSHDQSQQFLKKF
RAKSCLSYYLSWICCCGGGGGGGSGGWCPPSLQLKRPAAPSCSCAPRLRKLCCCCCCCRCRVVYAGGAGGCGCCAPCPRCSCDCTCA
CPRCCSCPRCSAACCCAPRCCLCL

>*S.bicolor_Gγ*4 coding sequence
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TTTGTCAGTGCAAAGACCGATCCAATGATACCAGTAAGTAAAAGGAAACATGGATCTTGCAGCTTCTCCCGGTGGATCAGATCAAAA
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GTGTGCTCCTGCTCCAGGTGCAAAGGAGGATGCTGCCACTGCCCGTCCTGTGGTAATAATCCCTGCTGTGCCGGTGGATGCTTATGC
TAG

>*S.bicolor_Gγ*4 translation
MGEAPQPKSPPRYPDLCGRRRLQLEVQILNREVGFLEQEIRGLERIQPVSRCCKDVNEFVSAKTDPMIPVSKRKHGSCSFSRWIRSK
LRTCFSCLCCFSCSCCTCRATQCCTPPTCSCPKTPSCSSCCCSPGWCSCSLPSCSCTGCGHCRTQCGNCCPSGCSCADCPCSCSCPS
CVSCCPGCFSCAGCSAGCLGALNRCLSCVSSCCSGMRPSCCKCQSSCCEAGSSSSSCRGTGTGACCRGSCLGAPAATPSCPECSCGC
VCSCSRCKGGCCHCPSCGNNPCCAGGCLC

>*S.bicolor_Gγ*5 coding sequence
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>*S.bicolor_Gγ*5 translation
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CNLSCCNPNCSSCCTCNPSCCKPNCNSCCRPNCSSCCNPSCCKPNCGSWWKPSCSCFKAPSCCKLQCSPNCCTCSLPRCSGCNPCGS
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>*Z.mays_Gα* coding sequence
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GTTCCAGAGAAGGAACTTGA

>*Z.mays_Gα* translation
MGSSCSRSHSFDEAEAAENAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIKLLFQTGFDEAELRSYTSVIHANVYQTI
KILYEGAKELAQVEPDSSKYVLSPDNQEIGEKLSEIGARLEYPSLNKERVQDVRKLWQDPAIQETYSRGSILQVPDCAQYFMENLDK
LSEEDYVPTKEDVLHARVRTNGVVETQFSPLGESKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAVIFCAAISEYDQMLFEDETKNR
MMETKELFDWVLKQRCFEKTSFMLFLNKFDIFERKIQKVPLSVCEWFKDYQPTAPGKQEVEHAYEFVKKKFEELYFQSSKPDRVDRV
FKIYRTTALDQKLVKKTFKLIDESMRRSREGT

>*Z.mays_Gβ* coding sequence
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>*Z.mays_Gβ* translate
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TSSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDTTVRLWDIRIASRAVRTYHGHEDDVNSVKFFPDG
HRFGTGSDDGTCRLFDMRTGHQLQVYSREPDRNSNELPTVTSIAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGNLQNSHDGRIS
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>*Z.mays_Gγ*1 coding sequence
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AGAAAAGCTGACCCTCTACTTCCTGTATCTGCTGGACCTGTGAATCAGTCCTGGGATAGGTGGTTTGAAGGTCCTCAAGATCTGCGC
GGATGCAAATGCTGGTTTTTGTGA

>*Z.mays_Gγ*1 translation
MQVGDGGGDSADLRGRHRIQAELKKLEQEARFLEEELEELDKADKVSSALQEFLIAMERKADPLLPVSAGPVNQSWDRWFEGPQDLR
GCKCWFL

>*Z.mays_Gγ*2 coding sequence
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CAGCAGCAGCCGCGCCCGCCGCCGCCGCTCGCCAGGAACGTCGGCTACGTCGGCAAGCACCGCCTCTCCGCCGCCATCGCCCGCTTC
GACCAGGAGCTCCAGTCCCTCCAGGACGAGCTGGACGAGCTTGAGACCATGGAATCTGCGTCCGCGGCGTGCCAGGAAGTGGTCACG
AGTACGGAAGGGAAGCCTGACCCGCTTCTTCCTGTCACCAGTGGCCCGGAGAACTCGTCTTGGGACAGGTGGTTCCAGCGGGTGCGC
TCCCGCAGCAACAAATGGTGGGCATCGAAAGGCCCTGACTTCTCGTAG

>*Z.mays_Gγ*2 translation
MRGQANGVEDRRQRGDDHEADNDGEEAEEEEGDDEGRHRGQGPPQQRRHQAQRPYSGPQQQPRPPPPLARNVGYVGKHRLSAAIARF
DQELQSLQDELDELETMESASAACQEVVTSTEGKPDPLLPVTSGPENSSWDRWFQRVRSRSNKWWASKGPDFS

>*Z.mays_Gγ*3 coding sequence
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GATGAGTTCGTTGGAAGGAACCCGGATCCGTTCCTAACGATTCAGCAAGAGAGAGGAAGCCACGATCAATCTCAGCAGTTTCTGAAG
AAGTTCCGAGGAAAGAGCTGCCTGAGCTACTACCTCTCGTGGATCTGCGGTGGCGGGTGGTGGTGCCCGCCGCCGTTGCAGCTCAAG
AGGCCACCGGCGCCGAGCTGCTCCTGCGCGCCGCGGCTGGGGAAGCTCTGCTCCTCCACCGCCTCCTCCTGCTGCAGCTGTTGCTGC
TGCCGGTTCCGCGTCGTGTACGCCGCCGCCGGCTGCGGGTGCTGCGCGCCGTGCCCGCGCTGCTCGTGCGACTGCACCTGCGCCTGC
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>*Z.mays_Gγ*3 translation
MAAAAAPRPKSPPASPDPCGRHRLQLAVDALHREIGFLEGEISSIEGVHAASRCCKEVDEFVGRNPDPFLTIQQERGSHDQSQQFLK
KFRGKSCLSYYLSWICGGGWWCPPPLQLKRPPAPSCSCAPRLGKLCSSTASSCCSCCCCRFRVVYAAAGCGCCAPCPRCSCDCTCAC
PRCCSCACPMCSAACCAPRCCLCL

>*Z.mays_Gγ*4 coding sequence
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GGGGGTTCGTCCTGCCGCGGCACCGGCACGGGAGCGTGCTGCCGCGGTTCGTGCCTCGGTGCCCCGGCGTCGTCGTGCCCGGAGTGC
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TGCTTGTGCTAG

>*Z.mays_Gγ*4 translation
MGEAPQPKSPPRYPDLCGRRRLQLEVQILNREVGFLEQEIQGLERIQPVSRCCNDVNEFVSAKTDPMIPVSKRRHGSCSFSRWIRSK
LRTCFSCLCCWCHCLPKPNAPSCFSCSCCTCRDTQCCTPICRCSKTPSCSPGCCTCSLPSCSCKTPPGCGHCRPQCSSCCSSGCSCA
DCPCSCSCPPCCSCPGFFSCEGCSAGCLGALNRCLGGLSSCCSEMRPSCCKCQSSCCEGGSSCRGTGTGACCRGSCLGAPASSCPEC
SCGCVCSCSRCKGGCRCPSCGSNPCCPGGCLC

>*Z.mays_Gγ*5 coding sequence
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CAGTCTCTAGAAGTTGTAAAGAGGTAAATGAGTTTGTTGGTACAAAACAAGACCCACTAATACCGATAAAGAAGAAGACGCACAGAT
CATGCCGTCTTTTTTGGTGGATCAGATCAAAATTGTGCATATGCGTGTCATGGTTCTGTTGCTCCTGCCATTGCTTGCCCAGCTGCA
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AACCGAACTGCAGCTGCTTCAAGACCCTCTCGTGCTGCAAATTCCAGTGCAGCCCAAACTGCTGCACCTGCAGCCTCCCGAGTTGCT
CCGGCTGCAACCCCTGCGGCAGTTGCAAGAAATGCTGGTCATGCCCCAGCGACTGCAAGCCAAGCTGTGGCTGTTTCAGCGCGCAAT
GTTGCAGCTGCGCGGGATGCTGCTCGTGCTCAAGCTGTTTCAGTTGCTTCGGTTGTTTCAAGTCCTTCAAGTGCTCCAACCTGTTTG
GGTGCTGCTCCTGCAAGCAGTGCTTCAAGTGCCAGTCCTCGTGCTGCAAGGGGGCGTCGTCCTGCTGCAAATGCCAGTCGTCGTGCT
GTGAGGGTGAAGACGGCAGCAGCTGCTGCCGGAGGCCATGCTGCACTTTTCCGAAGCCAGCTTGCTCTGGGTGTTCGTGCGGGTGCG
CTTGGTCCTGCAGAAAGTGTACAGAATGGTGTCGATGTTCCGGGTGCCGCAATCCGTGCTGTGCCACTGGAT
GCTTGTGTTGA

>*Z.mays_Gγ*5 translation
MGEEVAVVLEPPRPKSPPRYPDLCGRRRLQLELQALNREIDFLKDELQSLEGVPPVSRSCKEVNEFVGTKQDPLIPIKKKTHRSCRL
FWWIRSKLCICVSWFCCSCHCLPSCKRPCCLDCSCCSCPDLSCCKPSCKSCNKPCFGPNSCSCCDISCCKPDCPSCSSNCSSCCTGS
PSCCKPNCNTCCRPNCSSCCDPSCCKPNCSCFKTLSCCKFQCSPNCCTCSLPSCSGCNPCGSCKKCWSCPSDCKPSCGCFSAQCCSC
AGCCSCSSCFSCFGCFKSFKCSNLFGCCSCKQCFKCQSSCCKGASSCCKCQSSCCEGEDGSSCCRRPCCTFPKPACSGCSCGCAWSC
RKCTEWCRCSGCRNPCCATGCLC

>*O.sativa_Gα* coding sequence
ATGGGCTCATCCTGTAGCAGATCTCATTCTTTAAGTGAGGCTGAAACAACCAAAAATGCAAAATCTGCAGACATTGACAGGCGAATT
TTGCAAGAGACAAAAGCAGAGCAACACATCCACAAGCTCTTACTTCTTGGTGCGGGAGAATCAGGGAAGTCTACGATATTTAAACAG
ATTAAGCTCCTTTTCCAAACTGGCTTTGATGAGGCAGAACTTAGGAGCTACACATCAGTTATCCATGCAAACGTCTATCAGACAATT
AAAATACTATATGAAGGAGCAAAAGAACTCTCACAAGTGGAATCAGATTCCTCAAAGTATGTTATATCCCCAGATAACCAGGAAATT
GGAGAAAAACTATCAGATATTGATGGCAGGTTGGATTATCCACTGCTGAACAAAGAACTTGTACTCGATGTAAAAAGGTTATGGCAA
GACCCAGCCATTCAGGAAACTTACTTACGTGGAAGTATTCTGCAACTTCCTGATTGTGCACAATACTTCATGGAAAATTTGGATCGA
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GATATATTCGAGAAGAAAATACAAAAGGTTCCTTTAAGTGTGTGCGAGTGGTTTAAAGACTACCAGCCTATTGCACCTGGGAAACAG
GAGGTTGAACATGCATATGAGTTTGTCAAGAAGAAGTTTGAAGAGCTCTACTTCCAGAGCAGCAAGCCTGACCGTGTGGACCGCGTC

TTCAAAATCTACAGAACTACGGCCCTAGACCAGAAACTTGTAAAGAAGACATTCAAGTTGATTGATGAGAGCATGAGACGCTCCAGG
GAAGGAACTTGATTCAGAGCTAAGACTAGGTTGTAA

>*O.sativa_Gα* translation
MGSSCSRSHSLSEAETTKNAKSADIDRRILQETKAEQHIHKLLLLGAGESGKSTIFKQIKLLFQTGFDEAELRSYTSVIHANVYQTI
KILYEGAKELSQVESDSSKYVISPDNQEIGEKLSDIDGRLDYPLLNKELVLDVKRLWQDPAIQETYLRGSILQLPDCAQYFMENLDR
LAEAGYVPTKEDVLYARVRTNGVVQIQFSPVGENKRGGEVYRLYDVGGQRNERRKWIHLFEGVNAVIFCAAISEYDQMLFEDETKNR
MMETKELFDWVLKQRCFEKTSFILFLNKFDIFEKKIQKVPLSVCEWFKDYQPIAPGKQEVEHAYEFVKKKFEELYFQSSKPDRVDRV
FKIYRTTALDQKLVKKTFKLIDESMRRSREGT

>*O.sativa__Gβ* coding sequence
ATGGCGTCCGTGGCGGAGCTCAAGGAGAAGCACGCGGCGGCCACGGCGTCGGTGAACTCCCTGCGGGAGCGGCTCCGTCAGAGGCGG
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GGAAGGCTAATTGTATGGAATGCATTAACAAGTCAAAAAACACATGCCATAAAGTTACATTGCCCATGGGTGATGACATGTGCATTT
GCACCCAATGGCCAATCTGTTGCCTGTGGTGGTCTTGACAGCGCATGCTCTATCTTCAATCTTAACTCACAAGCAGACAGAGATGGG
AATATACCAGTATCAAGAATACTTACCGGACACAAAGGCTATGTTTCATCCTGTCAGTATGTCCCAGATCAGGAAACCCGCCTAATT
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CAGTCGGGAACCTGATAGAAATGATAATGAACTCCCAACTGTTACATCTATTGCATTTTCGATATCAGGAAGGCTTCTTTTTGCTGG
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GCGTATAAGCTGCCTTGGTCTTTCTTCTGATGGGAGTGCATTGTGTACAGGAAGTTGGGACAAGAATTTGAAGATTTGGGCCTTCAG
CGGACACCGGAAAATAGTTTGA

>*O.sativa__Gβ* translation
MASVAELKEKHAAATASVNSLRERLRQRRQMLLDTDVERYSRTQGRTPVSFNPTDLVCCRTLQGHSGKVYSLDWTPEKNWIVSASQD
GRLIVWNALTSQKTHAIKLHCPWVMTCAFAPNGQSVACGGLDSACSIFNLNSQADRDGNIPVSRILTGHKGYVSSCQYVPDQETRLI
TSSGDQTCVLWDVTTGQRISIFGGEFPSGHTADVLSLSINSSNSNMFVSGSCDATVRLWDIRIASRAVRTYHGHEGDINSVKFFPDG
QRFGTGSDDGTCRLFDVRTGHQLQVYSREPDRNDNELPTVTSIAFSISGRLLFAGYSNGDCYVWDTLLAEVVLNLGNLQNSHEGRIS
CLGLSSDGSALCTGSWDKNLKIWAFSGHRKIV

>*O.sativa__Gγ*1 coding sequence
ATGCAGGCCGGAGGAGGAGGGGACGCCGGGGACACGCGGGGGCGGCACCGGATCCAGGCGGAGCTCAAGAAGCTCGAGCAAGAGGCG
CGCTTTCTCGAGGAGGAACTTGAAGAGCTTGATAAAACGGATAAGGTGTCAGCAGCATTGCAAGAGCTTATGGTAACAGCGGAGAGC
AAAGCGGATCCTCTACTTCCAGTGACTACTGGGCCTGCTTGCCAGTCTTGGGACAGATGGTTTGAAGGTCCGCAAGATCTGCGCAGA
TGCAAATGCTGGTTTTTGTGA

>*O.sativa__Gγ*1 translation
MQAGGGGDAGDTRGRHRIQAELKKLEQEARFLEEELEELDKTDKVSAALQELMVTAESKADPLLPVTTGPACQSWDRWFEGPQDLRR
CKCWFL

>*O.sativa__Gγ*2 coding sequence
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AGGAGCGTGGGGTACGTCGGGAAGCACCGCCTCTCCGCCGCCATCGCCCGCCTCGACCAGGAGCTCCAGTCGCTGCAGGATGAACTG
AACGAGCTTGAAACCATGGAACCGGCATCTGCTGCATGCCAAGGAGTGATCACAAGTACAGAGGGAAAATCCGACCCGCTTCTTCCT
GTCACCATTGGTCCAGAGAACGCTTCTTGGGAGAGATGGTTTCAGCGCGTGCGTAGCTCTTGCAGTAACAAATGGTGGGCATCCAAA
GGCTCAGATTTTCCCTAG

>*O.sativa__Gγ*2 translation
MRGEANGEEEQQPPRRNHLRDDAEEEEEVERRAARPVSGQQQQQQRRRPTDVGGGAAMRSVGYVGKHRLSAAIARLDQELQSLQDEL
NELETMEPASAACQGVITSTEGKSDPLLPVTIGPENASWERWFQRVRSSCSNKWWASKGSDFP

>*O.sativa__Gγ3* coding sequence
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CGCTGTTTGTGCAGAGCAAGTGCGTGCTGCCTCAGCTACCTCTCCTGGATCTGCTGCTGCAGCAGCGCCGCCGGCGGCTGCTCATCC
TCCTCCTCCTCCTCCTTCAACCTCAAGAGGCCGAGCTGCTGCTGCAACTGCAACTGCAACTGCTGCTCCTCCTCCTCCTCCTCATGT
GGGGCGGCGTTAACGAAGAGTCCGTGTCGCTGCCGCCGCCGCAGCTGCTGCTGCCGTCGCTGCTGCTGCGGCGGCGTCGGCGTCCGC
GCGTGCGCGAGCTGCAGCTGCTCCCCGCCGTGCGCGTGCTGCGCGCCGCCGTGCGCGGGATGCTCGTGCCGCTGCACCTGCCCGTGC
CCGTGCCCCGGCGGCTGCTCCTGCGCGTGCCCGGCGTGCAGGTGCTGCTGCGGCGTCCCTCGTTGCTGCCCCCCCTGCTTGTGA

>*O.sativa__Gγ2* translation
MAAAPRPKSPPAPPDPCGRHRLQLAVDALHREIGFLEGEINSIEGIHAASRCCREVDEFIGRTPDPFITISSEKRSHDHSHHFLKKF
RCLCRASACCLSYLSWICCCSSAAGGCSSSSSSSFNLKRPSCCCNCNCNCCSSSSSSCGAALTKSPCRCRRRSCCCRRCCCGGVGVR
ACASCSCSPPCACCAPPCAGCSCRCTCPCPCPGGCSCACPACRCCCGVPRCCPPCL

>*O.sativa__Gγ4* coding sequence
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AACCGGGAGGTCGGCTTCCTCGAGCAAGAGCTACAGGGACTTGAACGGATTCAGCCGGTCTCGAGGTGTTGCAAAGATGTCAACGAA
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TGCCTCGGAGGCGCGACGCCGGCGCCGTCGTGCCCCGAGTGCTCCTGCGGCTGCTCGTGCTCCTGCCCGAGGTGCAAAGATGGGTGC
AGCCGCCCGTCGTGTGGCAATCCGTGCTGTGCCGGCGGATGCTTATGCTGA

>*O.sativa__Gγ4* translation
MGEAPRPKSPPRYPDLCGRRRLQLEMQILNREVGFLEQELQGLERIQPVSRCCKDVNEFVGAKSDPLIPINKRKHRSCSLYRWIRSK
LCNCLLCLCCWCRCLPKPKKPSCFSCSCCSCCDTSCCRPSCGCLKAPSSCCCKSNCSCCSSDCCTCSLPSCGCTGCGHCRPLCGGGG
GCCPPSDCCSSCKCSCSSCTRCCSSCAGGCKPSCSGCGTGCSSCGGGCCPKCSSCAAPCVGCLALLRRWLSCRSSCCKGQPSCCKCQ
SSCCEGEPSCCCCCGGGKGSSACCCGRPCCLGGATPAPSCPECSCGCSCSCPRCKDGCSRPSCGNPCCAGGCLC

>*O.sativa__Gγ5* coding sequence
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GGTTGCAACGGCTGTGGCCTTCCAAGCTGCGGTTGCAACGGCTGCGGCTCGTGCTCTTGCGCCCAATGCAAACCCGATTGTGGCTCG
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TCCTGCTGCGAGGGACACTGCTGCAGCCTCCCGAAACCGTCGTGCCCTGAATGTTCCTGTGGGTGTGTCTGGTCTTGCAAGAATTGT
ACAGAGGGTTGTCGATGCCCACGGTGTCGTAACCCATGCTGTCTCAGTGGTTGCTTATGTTGA

>*O.sativa__Gγ5* translation
MGEEAVVMEAPRPKSPPRYPDLCGRRRMQLEVQILSREITFLKDELHFLEGAQPVSRSGCIKEINEFVGTKHDPLIPTKRRRHRSCR
LFRWIGSKLCICISCLCYCCKCSPKCKRPRCLNCSCSSCCDEPCCKPNCSACCAGSCCSPDCCSCCKPNCSCCKTPSCCKPNCSCSC

PSCSSCCDTSCCKPSCTCFNIFSCFKSLYSCFKIPSCFKSQCNCSSPNCCTCTLPSCSCKGCACPSCGCNGCGCPSCGCNGCGCPSC
GCNGCGLPSCGCNGCGSCSCAQCKPDCGSCSTNCCSCKPSCNGCCGEQCCRCADCFSCSCPRCSSCFNIFKCSCAGCCSSLCKCPCT
TQCFSCQSSCCKRQPSCCKCQSSCCEGQPSCCEGHCCSLPKPSCPECSCGCVWSCKNCTEGCRCPRCRNPCCLSGCLC

Supplementary Table S2.8. Exon/Intron structure of G protein gene family members in *Triticum aestivum*

Gene	Exon(E)/Intron(I) lengths												
	Chr	**E1**	**I1**	**E2**	**I2**	**E3**	**I3**	**E4**	**I4**	**E5**	**I5**	**E6**	**I6**
	7AS	63	109	73	314	38	106	90	167	78	560	108	86
	1B	63	119	73	235	38	91	90	148	78	739	108	81
	7DS	63	92	73	286	38	100	90	164	78	921	108	82
GA1	**E7**	**I7**	**E8**	**I8**	**E9**	**I9**	**E10**	**I10**	**E11**	**I11**	**E12**	**I12**	**E13**
	102	129	56	105	135	85	94	107	140	97	172	NA	NA
	102	417	56	87	135	485	94	89	60	103	80	80	127
	102	118	56	109	135	88	94	93	140	93	172	NA	NA
	Chr	**E1**	**I1**	**E2**	**I2**	**E3**	**I3**	**E4**	**I4**	**E5**	**I5**	**E6**	
	4A	109	91	95	1553	425	634	364	1020	114	481	36	
Gβ	4B	109	107	95	1579	425	498	364	1032	114	436	36	
	4D	109	101	95	1638	425	534	364	1028	114	485	36	
	Chr	**E1**	**I1**	**E2**	**I2**	**E3**	**I3**	**E4**	**I4**	**E5**			
	5AL	114	2640	53	121	45	104	85	NA	NA			
Gγ1	5BL	114	3372	53	125	45	105	85	NA	NA			
	5DL	114	2670	50	121	45	102	85	NA	NA			
	6AS	225	4131	53	83	45	100	103	NA	NA			
Gγ2	6BS	240	4197	53	84	45	98	103	NA	NA			
	6DS	237	4184	53	84	45	99	103	NA	NA			
	7AS	99	393	53	711	45	82	54	287	259			
Gγ3	4AL	99	406	53	651	45	82	54	280	262			
	7DS	99	650	53	833	45	82	54	277	259			
	5AL	132	1135	59	625	45	78	45	385	577			
Gγ4	5BL	132	1244	59	567	45	79	45	281	577			
	5DL	132	1164	59	573	45	78	45	258	619			

Note: E and I denote exon and intron numbers resp. Chr is the chromosome location for gene copies. E/I lengths are determined by using genomic sequence from IWGSC with respective coding regions without 5' and 3' UTR regions. NA- Not available.

Supplementary Table S2.9. Determination of exons for monocot species and Arabidopsis

Genes	Number of exons								
	A. tauschii	***H. vulgare***	***S. cereale***	***B. distachyon***	***O. sativa***	***S. italica***	***Z. mays***	***S. bicolor***	***A. thaliana***
GA1	12	13	13	13	13	13	13	13	13
Gβ	6	6	6	6	6	6	6	6	6
Gγ1	4	4	4	4	4	4	4	4	4
Gγ2	4	5	4	4	4	4	4	4	4
Gγ3	5	5	5	5	5	5	5	5	5
Gγ4	5	5	NA	5	5	5	5	5	NA
Gγ5	NA	NA	NA	NA	5	NA	5	5	NA

Note: The number of exons for respective monocot species are determined by comparison of G protein genes coding regions for respective species with whole genome shotgun (WGS) sequences available at NCBI database.

Supplementary Table S2.10. Tissue specific expression for G protein gene family members in *Triticum aestivum* across seventy one tissues of Azhurnaya spring wheat

Supplementary Table S2.10-A. Tissue specific expression for *GA1* copies

Tissue	*GA1-A*		*GA1-B*		*GA1-D*	
	RPKM	SD	RPKM	SD	RPKM	SD
First leaf sheath - Tillering stage	5.92	0.41	5.23	0.94	11.68	0.5
Internode #2 - Milk grain stage	6.2	0.45	5.9	0.58	19.76	3.17
Shoot apical meristem - Seedling stage	18.47	2.32	16.97	0.69	34.92	1.23
Grain - Milk grain stage	5.23	0.74	5.97	1.15	23.34	0.83
First leaf blade - Seedling stage	3.19	2.68	2.54	1.5	11.76	5.81
Flag leaf blade - Full boot	2.05	0.5	2.59	0.19	7.22	0.56
Awn - 50 percent spike	10.86	2.11	8.94	1.85	26.92	4.42
flag leaf blade night (-0.25h) 06:45	3.51	1.02	3.36	0.33	8.44	0.8
Shoot axis - Flag leaf stage	13.73	3.11	15.15	2.27	33.37	0.71
Fifth leaf blade - Flag leaf stage	2.15	1.27	2.3	1.02	7.3	1.09
Third leaf sheath - Three leaf stage	19.12	0.9	14.83	1.03	35.01	1.62
Internode #2 - Ear emergence	4.9	0.82	5.02	0.71	14.45	1.98
Anther	1.13	0.43	2.62	0.8	3.65	1.39
Spike	18.47	0.66	15.52	2.28	36.57	1.96
Coleoptile	10.96	1.93	7.4	1.22	29.27	1.96
Stigma and Ovary	11.49	0.6	12.04	0.6	27.35	2.95
Roots - Flag leaf stage	7.57	0.16	7.23	0.46	26	2.35
Fifth leaf sheath - Flag leaf stage	5.46	1.59	3.05	0.43	21.11	9.65
Root apical meristem - Three leaf stage	13.21	0.97	11.17	1.15	33.25	2.92
Flag leaf sheath - Ear emergence	11.98	2.7	3.92	0.07	7.46	0.1
Roots - Three leaf stage	11.71	5.07	11.95	1.81	27.05	4.17
Axillary roots - Three leaf stage	11.26	1.39	8.98	1.34	32.91	3.69
Flag leaf sheath - 50 percent spike	4.43	0.71	3.73	1.08	6.4	0.95
Radicle - Seedling stage	11.7	0.31	9.32	0.8	33.43	1.03
Roots - 50 percent spike	6.17	1.23	7.12	1.31	19.45	4.79
Third leaf blade - Three leaf stage	3.31	3.77	2.26	1.91	10.73	8.74
Spikelets - 50 percent spike	8.47	1.41	7.07	0.98	22.95	2.22
Root apical meristem - Tillering stage	12.32	0.26	13.05	0.3	26.75	2.16
Grain - Ripening stage	2.02	0.28	4.8	0.65	17.15	4.12
Awns - Ear emergence	2.37	0.57	2.88	0.64	5.64	0.84
Glumes	2.94	0.64	5.34	0.53	7.31	0.5
Glumes - Ear emergence	2.62	0.28	4.34	0.26	7.58	0.33
Leaf ligule	3.92	0.29	4.3	0.53	8.91	0.94
Flag leaf blade - 50 percent spike	3.6	1.34	2.63	0.52	7.9	0.44
Internode #2 - 50 percent spike	4.51	0.31	4.84	0.1	17.75	1.41
Fifth leaf sheath - Fifth leaf stage	18.04	0.68	14.93	0.25	31.84	1.95
fifth leaf blade night (-0.25h) 21:45	2.06	0.26	3.78	0.62	21.37	3.68

Grain - Soft dough	2.74	0.69	3.92	0.41	14.75	2.87
Flag leaf blade (senescence) - Dough stage	5.45	0.19	3.92	0.05	9.08	1.17
Flag leaf blade night (-0.25h) 06:45 - Flag leaf stage	0.49	0.35	1.44	0.52	4.72	2.43
Flag leaf blade (senescence) - Ripening stage	4.43	0.18	5.62	1.03	13.5	2.78
First leaf blade - Tillering stage	5	1.57	3.86	0.47	9.88	0.4
Shoot apical meristem - Tillering stage	13.68	3.35	13.38	1.03	31.66	1.66
Shoot axis - First leaf stage	9.86	1.84	6.18	1.66	27.95	3.49
Roots - Seedling stage	10.85	0.73	8.45	1.01	32.1	1.87
Shoot axis - Milk grain stage	6.78	1.57	8.18	3.06	26.57	4.58
Fifth leaf blade - Fifth leaf stage	3.4	4.48	3.12	3.13	9.68	8.16
Flag leaf blade - Ear emergence	7.21	0.14	3	0.18	6.79	0.65
flag leaf blade night (+0.25h) 07:15	0.68	0.08	1.17	0.13	4.46	1.03
Fifth leaf blade night (-0.25h) 21:45	5.91	1.27	6.16	0.66	22.93	2.42
Shoot axis - Tillering stage	14.34	3.3	14.57	2.84	32.84	1.89
Stem axis - First leaf stage	9.86	1.84	6.18	1.66	27.95	3.49
Endosperm	0.99	0.34	2.46	0.28	4.94	1.13
Peduncle	4.32	1.09	6.01	0.03	9.96	0.4
Peduncle - 50 percent spike	3.75	3.32	3.06	0.93	18	10.6
Peduncle - Ear emergence	2.3	0.7	2.34	0.41	13.24	3.77
Flag leaf sheath - Full boot	2.88	1.48	2.42	0.55	7.16	0.8
Flag leaf blade - Flag leaf stage	0.99	0.41	1.35	0.55	6.14	1.02
Lemma	3.1	0.44	4.85	0.17	7.2	0.5
Lemma - Ear emergence	3.29	0.04	4.44	0.35	8.96	0.77
Awns - Milk grain stage	4.15	0.73	4.75	0.67	7.01	0.5
fifth leaf blade night (+0.25h) 22:15	1.72	0.58	3.34	1.02	18.93	1
Flag leaf blade - Milk grain stage	9.79	4.48	4.4	1.52	9.07	1.54
Grain - Hard dough	1.36	0.44	4.95	1.44	14.99	4.27
Flag leaf sheath - Milk grain stage	8.86	2.7	4.58	0.22	8.86	0.31
Embryo proper	3.7	0.4	6.92	0.76	27.25	2.69
Fifth leaf blade (senescence) - Milk grain stage	9.16	3.08	4.13	0.96	9.6	0.87
Roots - Tillering stage	10.06	0.64	11.09	1.35	26.32	1.7
Shoot axis - Full boot	13.07	1.89	13.9	1.58	36.33	1.85
Fifth leaf blade - Ear emergence	6.28	2.45	2.87	1.15	7.25	1.65
First leaf sheath - Seedling stage	8.68	4.9	5.69	3.61	26.14	5.31

Note: Tissue specific expression for G protein gene families across a panel of seventy one tissues in Azhurnya spring wheat is given. The expression values are represented as reads per kilo base per million (RPKM) based on three replicates each. eFP browser for *T. aestivum* is at bar Toronto (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi).

Supplementary Table S2.10-B. Tissue specific expression for *Gβ* copies

Tissue	*Gβ-A*		*Gβ-B*		*Gβ-D*	
	RPKM	SD	RPKM	SD	RPKM	SD
First leaf sheath - Tillering stage	7.91	0.44	6.4	0.41	8.19	0.47
Internode #2 - Milk grain stage	10.2	1.71	8.75	0.34	10.4	0.06
Shoot apical meristem - Seedling stage	13.09	1.39	11.92	0.77	12.98	0.66
Grain - Milk grain stage	7.49	0.47	6.7	0.43	10.02	0.56
First leaf blade - Seedling stage	4.17	2.19	5.66	2.2	6.88	2.54
Flag leaf blade - Full boot	2.73	0.47	4.11	0.31	5.35	0.4
Awn - 50 percent spike	9.2	0.25	10.74	0.1	11.72	0.17
flag leaf blade night (-0.25h) 06:45	4.25	0.67	4.75	0.23	6.07	0.52
Shoot axis - Flag leaf stage	12.88	2.43	10.8	1.76	12.52	2.26
Fifth leaf blade - Flag leaf stage	2.82	0.75	4.46	0.83	5.01	0.97
Third leaf sheath - Three leaf stage	10.94	0.33	11.93	0.18	10.5	1.07
Internode #2 - Ear emergence	7.13	0.48	7.08	0.77	8.5	0.67
Anther	1.47	0.03	2.86	0.34	2.73	0.38
Spike	12.3	1.31	12.72	0.64	13.74	0.29
Coleoptile	7.62	0.49	8.68	0.48	10.78	0.42
Stigma and Ovary	14.42	0.68	12.74	1.65	14.48	0.58
Roots - Flag leaf stage	10.3	0.26	8.87	0.64	11	0.55
Fifth leaf sheath - Flag leaf stage	6.1	0.98	8.35	2.52	9.56	1.36
Root apical meristem - Three leaf stage	9.26	0.43	8.7	0.86	10.54	0.92
Flag leaf sheath - Ear emergence	6.2	0.76	5.15	0.45	7.32	0.96
Roots - Three leaf stage	10.97	0.82	9.85	1.01	10.5	0.6
Axillary roots - Three leaf stage	10.1	1.24	9	0.45	11.13	0.6
Flag leaf sheath - 50 percent spike	4.77	0.29	4.68	0.44	6.14	0.46
Radicle - Seedling stage	9.02	0.29	9.33	0.32	11.01	0.59
Roots - 50 percent spike	9.7	2.68	8.83	3.13	11.01	3.1
Third leaf blade - Three leaf stage	2.91	2.12	4.18	2.78	3.88	2.39
Spikelets - 50 percent spike	7.62	0.53	8.98	0.5	10.26	0.73
Root apical meristem - Tillering stage	9.76	0.54	10.07	0.17	11.18	0.51
Grain - Ripening stage	8.19	2.85	4.39	0.58	5.48	0.38
Awns - Ear emergence	4.47	1	4.54	1.18	5.39	1.33
Glumes	6.89	0.27	5.86	0.19	8.03	0.34
Glumes - Ear emergence	6.4	0.19	6.49	1.07	8.28	1.05
Leaf ligule	5.58	0.67	5.72	0.5	7.06	0.62
Flag leaf blade - 50 percent spike	4.26	0.48	4.09	0.42	5.98	0.42
Internode #2 - 50 percent spike	7.96	0.38	7.69	1.41	8.98	0.79
Fifth leaf sheath - Fifth leaf stage	11.68	1.41	11.63	0.69	10.93	1.05
fifth leaf blade night (-0.25h) 21:45	4.38	1.06	4.65	0.45	5.29	0.3
Grain - Soft dough	5.45	1.08	4.49	1.33	6.51	1.73
Flag leaf blade (senescence) - Dough	5.54	0.36	4.48	0.49	7.65	0.48

stage						
Flag leaf blade night (-0.25h) 06:45 - Flag leaf stage	1.62	0.56	2.84	0.68	3.01	0.52
Flag leaf blade (senescence) - Ripening stage	9.05	0.01	7.78	1.29	10.5	0.01
First leaf blade - Tillering stage	6.26	2.16	5.5	1.45	7.54	2.08
Shoot apical meristem - Tillering stage	10.35	2.26	10.78	0.58	11.35	1.16
Shoot axis - First leaf stage	5.58	1.49	8.87	1.25	8.08	1.07
Roots - Seedling stage	9.63	0.2	9.07	0.56	11.04	1.06
Shoot axis - Milk grain stage	11.93	0.09	10.65	1.49	13.21	1.13
Fifth leaf blade - Fifth leaf stage	3.25	2.23	4.22	2.7	4.67	1.66
Flag leaf blade - Ear emergence	4.48	0.37	4.38	0.25	6.39	0.39
flag leaf blade night (+0.25h) 07:15	1.61	0.29	4	0.35	3.68	0.32
Fifth leaf blade night (-0.25h) 21:45	7.37	0.56	6.59	1.1	8.9	0.7
Shoot axis - Tillering stage	11.65	1.48	10.22	0.67	11.25	0.87
Stem axis - First leaf stage	5.58	1.49	8.87	1.25	8.08	1.07
Endosperm	4.24	0.8	2.48	0.56	3.51	0.65
Peduncle	7.66	0.83	5.74	0.48	8.26	0.96
Peduncle - 50 percent spike	4.79	1.09	7.37	2.93	7.85	1.88
Peduncle - Ear emergence	5.02	0.99	8.35	1.94	9.18	1.59
Flag leaf sheath - Full boot	3.07	0.9	4.34	1.17	5.12	1.01
Flag leaf blade - Flag leaf stage	1.86	0.34	3.46	0.68	3.96	0.57
Lemma	7.53	0.54	6.17	0.84	7.91	0.56
Lemma - Ear emergence	6.94	1.03	5.91	0.25	7.47	0.28
Awns - Milk grain stage	6.52	0.51	6.28	1.02	7.66	0.16
fifth leaf blade night (+0.25h) 22:15	4.23	0.99	3.76	0.72	4.8	0.52
Flag leaf blade - Milk grain stage	5.12	1.32	4.89	1.48	6.5	1.96
Grain - Hard dough	5.47	1.19	4.42	1.83	5.54	2.1
Flag leaf sheath - Milk grain stage	6.74	0.63	5.18	0.03	7.74	0.06
Embryo proper	7.3	0.37	5.16	0.51	7.07	0.58
Fifth leaf blade (senescence) - Milk grain stage	7.32	0.82	6.57	0.39	8.5	1.33
Roots - Tillering stage	9.81	0.79	9.13	0.73	11.15	0.86
Shoot axis - Full boot	10.89	0.47	10.39	0.48	10.82	1.03
Fifth leaf blade - Ear emergence	4.75	0.95	4.42	0.37	6.04	1.19
First leaf sheath - Seedling stage	7.08	2.76	8.23	2.02	8.85	2.36

Supplementary Table S2.10-C. Tissue specific expression for *Gγ1* copies

Tissue	*Gγ1-A*		*Gγ1-B*		*Gγ1-D*	
	RPKM	SD	RPKM	SD	RPKM	SD
First leaf sheath - Tillering stage	0.73	0.53	0.1	0.08	2.44	0.61
Internode #2 - Milk grain stage	0	0	0	0	0.14	0.09
Shoot apical meristem - Seedling stage	5.09	2.23	0	0.05	6.65	2.89
Grain - Milk grain stage	2.79	1.03	0	0.02	3.84	0.65
First leaf blade - Seedling stage	0.52	0.61	0	0.04	1.15	0.9
Flag leaf blade - Full boot	0	0	0	0	0.15	0.09
Awn - 50 percent spike	0.09	0.13	0	0	0.47	0.28
flag leaf blade night (-0.25h) 06:45	0	0	0	0	0.08	0.06
Shoot axis - Flag leaf stage	5.09	3.02	0	0.01	8.95	5.07
Fifth leaf blade - Flag leaf stage	0.32	0.45	0	0	0.08	0.03
Third leaf sheath - Three leaf stage	0.59	0.19	0	0.02	0.56	0.31
Internode #2 - Ear emergence	0.17	0.24	0	0	0.15	0.11
Anther	1.16	0.56	0	0.02	0.45	0.25
Spike	0.81	0.48	0.35	0.04	0.95	0.41
Coleoptile	0.65	0.74	0	0.02	1.45	0.31
Stigma and Ovary	0.48	0.11	0	0.06	0.11	0.06
Roots - Flag leaf stage	7.49	0.69	0	0.05	9.56	0.86
Fifth leaf sheath - Flag leaf stage	0.38	0.27	0	0.02	0.77	0.46
Root apical meristem - Three leaf stage	20.18	4.75	0	0.02	31.08	6.43
Flag leaf sheath - Ear emergence	0	0	0	0.03	0.29	0.19
Roots - Three leaf stage	16.19	8.49	0	0.04	20.06	12.36
Axillary roots - Three leaf stage	15.28	3.24	0	0	23.53	2.86
Flag leaf sheath - 50 percent spike	0.08	0.11	0	0	0.19	0.1
Radicle - Seedling stage	15.01	2.24	0	0	20.26	2.42
Roots - 50 percent spike	13.76	5.4	0	0	13.87	4.6
Third leaf blade - Three leaf stage	0	0	0	0	0.4	0.11
Spikelets - 50 percent spike	0.3	0.22	0	0.01	0.54	0.21
Root apical meristem - Tillering stage	13.24	2.86	0	0.02	16.87	1.11
Grain - Ripening stage	1.52	0.79	0.06	0.08	6.19	3.44
Awns - Ear emergence	0.06	0.08	0	0.02	0.49	0.21
Glumes	0	0	0.11	0.11	0.11	0.03
Glumes - Ear emergence	0	0	0	0	0.11	0.07
Leaf ligule	0	0	0	0	0.74	0.58
Flag leaf blade - 50 percent spike	0	0	0	0	0.16	0.09
Internode #2 - 50 percent spike	0.25	0.36	0	0	0.26	0
Fifth leaf sheath - Fifth leaf stage	0.17	0.12	0	0	0.22	0.1
fifth leaf blade night (-0.25h) 21:45	0	0	0	0	0.19	0.02
Grain - Soft dough	3.17	1.47	0	0	5.45	0.96
Flag leaf blade (senescence) - Dough	0	0	0	0	0.22	0.08

stage						
Flag leaf blade night (-0.25h) 06:45 - Flag leaf stage	0	0	0	0	0.31	0.16
Flag leaf blade (senescence) - Ripening stage	0	0	0	0.03	1.21	0.11
First leaf blade - Tillering stage	0	0	0	0.02	0.34	0.24
Shoot apical meristem - Tillering stage	6.53	3.93	0	0.04	9.45	3.86
Shoot axis - First leaf stage	0.56	0.08	0	0.02	1.06	0.67
Roots - Seedling stage	14.72	2.12	0	0	20.4	0.5
Shoot axis - Milk grain stage	1.91	0.98	0	0.06	1.41	0.71
Fifth leaf blade - Fifth leaf stage	0.15	0.11	0	0.02	0.25	0.06
Flag leaf blade - Ear emergence	0.08	0.12	0	0	0.06	0.04
flag leaf blade night (+0.25h) 07:15	0	0	0	0	0.31	0.09
Fifth leaf blade night (-0.25h) 21:45	0	0	0	0.06	0	0.05
Shoot axis - Tillering stage	9.27	2.14	0	0	11.39	0.97
Stem axis - First leaf stage	0.56	0.08	0	0.02	1.06	0.67
Endosperm	1.08	0.15	0.06	0.09	3.32	1.36
Peduncle	0.06	0.09	0	0	0.37	0.25
Peduncle - 50 percent spike	0	0	0.06	0.06	1.29	0.77
Peduncle - Ear emergence	0	0	0	0.02	0.85	0.34
Flag leaf sheath - Full boot	0	0	0	0	0.47	0.47
Flag leaf blade - Flag leaf stage	0	0	0	0	0.19	0.06
Lemma	0	0	0	0	0.33	0.11
Lemma - Ear emergence	0.28	0.4	0	0	0.21	0.13
Awns - Milk grain stage	0	0	0	0	0.67	0.47
fifth leaf blade night (+0.25h) 22:15	0.07	0.1	0	0	0.42	0.15
Flag leaf blade - Milk grain stage	0	0	0.09	0.08	0.06	0.05
Grain - Hard dough	0.67	0.49	0	0	3.49	0.89
Flag leaf sheath - Milk grain stage	0	0	0	0.06	0.08	0.07
Embryo proper	0.78	0.68	0	0.02	5.29	1.54
Fifth leaf blade (senescence) - Milk grain stage	0	0	0	0	0.05	0.04
Roots - Tillering stage	16.14	2.77	0.1	0.07	23.5	5.32
Shoot axis - Full boot	19.5	3.39	0	0	20.39	5.85
Fifth leaf blade - Ear emergence	0	0	0	0.02	0.17	0.16
First leaf sheath - Seedling stage	0.52	0.36	0	0	1.11	0.34

Supplementary Table S2.10-D. Tissue specific expression for *Gγ2* copies

Tissue	*Gγ2-A*		*Gγ2-B*		*Gγ2-D*	
	RPKM	SD	RPKM	SD	RPKM	SD
First leaf sheath - Tillering stage	7.75	0.52	14.62	2.48	13.67	1
Internode #2 - Milk grain stage	7.99	0.28	17.76	0.09	12.72	0.56
Shoot apical meristem - Seedling stage	6.44	0.58	9.55	0.18	9.06	0.81
Grain - Milk grain stage	4.56	0.87	3.92	0.66	4.33	0.45
First leaf blade - Seedling stage	4.06	1	6	0.96	4.57	1.14
Flag leaf blade - Full boot	5.98	0.65	11.7	2.2	9.85	0.21
Awn - 50 percent spike	5.19	0.61	7.35	0.78	7.44	1.26
flag leaf blade night (-0.25h) 06:45	23.29	3.28	42.13	5.81	32.09	5.11
Shoot axis - Flag leaf stage	7.03	0.53	10.4	0.6	10.82	0.61
Fifth leaf blade - Flag leaf stage	5.62	2.06	11.14	2.63	9.14	3.6
Third leaf sheath - Three leaf stage	5.27	0.46	7.06	0.83	6.42	0.44
Internode #2 - Ear emergence	6.72	0.95	16.05	2.48	10.93	1.82
Anther	2.52	0.15	3.8	1.05	3.21	0.78
Spike	4.98	0.24	8.18	0.75	7.08	0.81
Coleoptile	4.87	0.26	9.6	0.35	7.7	0.63
Stigma and Ovary	2.78	0.14	1.62	0.32	3.64	0.85
Roots - Flag leaf stage	10.78	1.37	16.57	0.5	13.35	0.11
Fifth leaf sheath - Flag leaf stage	6.17	0.7	11.12	0.66	7.56	0.57
Root apical meristem - Three leaf stage	9.68	0.76	14.19	0.57	12.32	1.12
Flag leaf sheath - Ear emergence	15.13	1.54	31.51	4.51	23.38	2.66
Roots - Three leaf stage	10.51	3.94	15.09	5.54	11.93	4.02
Axillary roots - Three leaf stage	8.44	1.09	14.44	2.02	12.34	1.38
Flag leaf sheath - 50 percent spike	9.96	0.64	20.26	5.24	14.98	1.64
Radicle - Seedling stage	7.56	0.04	12.6	0.7	11.34	1.01
Roots - 50 percent spike	10.89	3.55	15.28	4.41	13.45	2.69
Third leaf blade - Three leaf stage	3.71	1.05	7.17	1.38	5.97	1.52
Spikelets - 50 percent spike	5.15	1.21	8.49	1.37	8.29	0.8
Root apical meristem - Tillering stage	7.04	0.68	11.12	0.86	9.47	0.48
Grain - Ripening stage	12.02	5.54	8.54	3.62	9.97	2.5
Awns - Ear emergence	5	1.29	9.58	1.82	7.55	1.2
Glumes	7.59	0.38	14.57	0.63	11.58	1.14
Glumes - Ear emergence	6.75	0.26	12.42	1.25	9.16	1.34
Leaf ligule	5.84	0.29	11.18	1.02	9.89	0.12
Flag leaf blade - 50 percent spike	7.84	1.01	14.14	0.74	11.47	2.09
Internode #2 - 50 percent spike	8.73	0.64	16.83	2.22	12.36	2.61
Fifth leaf sheath - Fifth leaf stage	4.44	0.58	6.66	0.34	6.76	0.95
fifth leaf blade night (-0.25h) 21:45	3.87	0.63	7.61	0.71	7.61	1.47
Grain - Soft dough	5.74	1.17	3.24	0.5	4.8	0.19
Flag leaf blade (senescence) - Dough stage	11.73	0.69	18.36	0.49	13.84	0.53

Flag leaf blade night (-0.25h) 06:45 - Flag leaf stage	5.95	2.05	11.43	4.83	10.86	4.62
Flag leaf blade (senescence) - Ripening stage	8.87	2.6	16.46	1.07	12.73	2.87
First leaf blade - Tillering stage	10.85	1.8	16.07	2.13	13.83	0.81
Shoot apical meristem - Tillering stage	7.71	0.96	11.87	0.67	10.42	1.2
Shoot axis - First leaf stage	4.79	1.05	9.1	0.36	7.78	0.78
Roots - Seedling stage	8.31	0.62	14.77	1.61	11.77	1.24
Shoot axis - Milk grain stage	7.31	1.15	14.04	1.8	12.18	1.53
Fifth leaf blade - Fifth leaf stage	2.78	0.4	5.43	0.8	5.1	1.05
Flag leaf blade - Ear emergence	10.05	0.35	20.19	0.89	17.75	0.76
flag leaf blade night (+0.25h) 07:15	4.49	0.45	10.62	0.62	8.79	0.65
Fifth leaf blade night (-0.25h) 21:45	10.53	0.43	17.51	1.25	15.32	1.7
Shoot axis - Tillering stage	7.56	0.86	12.89	0.95	11.5	0.52
Stem axis - First leaf stage	4.79	1.05	9.1	0.36	7.78	0.78
Endosperm	6.74	0.3	3.84	0.61	5.18	0.33
Peduncle	7.04	0.22	16.86	2.1	11.84	0.77
Peduncle - 50 percent spike	4.18	1.04	7.41	0.96	5.97	1.45
Peduncle - Ear emergence	4.77	0.55	9.53	1.09	7	0.7
Flag leaf sheath - Full boot	6.47	1.37	13.4	4.22	10.29	2.64
Flag leaf blade - Flag leaf stage	3.54	0.68	7.66	1.12	6.47	0.28
Lemma	8.06	0.51	16.08	1.95	11.18	1.52
Lemma - Ear emergence	6.98	0.21	14.17	1.69	8.95	0.87
Awns - Milk grain stage	8.84	0.87	16.21	1.72	12.8	1.97
fifth leaf blade night (+0.25h) 22:15	3.55	0.78	7.4	0.85	7.05	1.93
Flag leaf blade - Milk grain stage	14.67	1.46	23.84	1.29	21.07	3.39
Grain - Hard dough	6.72	2.16	5.4	2.86	5.7	2.28
Flag leaf sheath - Milk grain stage	14.04	0.77	24.18	2.37	19.7	3.22
Embryo proper	9.75	1.25	5.14	0.98	6.67	0.69
Fifth leaf blade (senescence) - Milk grain stage	14.36	1.64	25.03	5.01	20.83	2.29
Roots - Tillering stage	8.42	0.97	14.08	1.74	11.77	0.25
Shoot axis - Full boot	7.12	0.29	13.98	1.16	11.24	1.43
Fifth leaf blade - Ear emergence	11.04	2.32	20.9	4.76	16.56	2.44
First leaf sheath - Seedling stage	5.03	0.93	8.53	0.12	6.82	0.11

Supplementary Table S2.10-E. Tissue specific expression for *Gγ3* copies

Tissue	*Gγ3-A*		*Gγ3-AL*		*Gγ3-D*	
	RPKM	SD	RPKM	SD	RPKM	SD
First leaf sheath - Tillering stage	0.24	0.05	1.26	0.2	0.85	0.53
Internode #2 - Milk grain stage	0.28	0.12	1.73	0.62	1.2	0.17
Shoot apical meristem - Seedling stage	1.7	0.02	1.53	0.16	2.14	0.18
Grain - Milk grain stage	0.58	0.27	1.64	0.11	1.44	0.69
First leaf blade - Seedling stage	0.39	0.29	1.04	0.5	0.65	0.44
Flag leaf blade - Full boot	0	0	0.15	0.04	0.05	0.04
Awn - 50 percent spike	1.25	0.29	2.44	0.65	1.65	0.29
flag leaf blade night (-0.25h) 06:45	0	0.02	0.21	0.11	0.09	0.01
Shoot axis - Flag leaf stage	2	0.28	1.76	0.26	2.2	0.51
Fifth leaf blade - Flag leaf stage	0.05	0.02	0.21	0.06	0.09	0.05
Third leaf sheath - Three leaf stage	1.47	0.24	0.97	0.18	1.08	0.27
Internode #2 - Ear emergence	0.1	0.07	1.71	0.47	0.86	0.34
Anther	0	0.03	0.24	0.06	0.06	0.06
Spike	1.25	0.26	1.57	0.13	1.67	0.33
Coleoptile	0.89	0.29	1.56	0.37	1.23	0.22
Stigma and Ovary	1.7	0.21	2.39	0.41	1.92	0.52
Roots - Flag leaf stage	0.44	0.15	1.62	0.43	1.4	0.45
Fifth leaf sheath - Flag leaf stage	0.67	0.44	1.66	0.94	0.61	0.25
Root apical meristem - Three leaf stage	1.42	0.13	2.93	0.48	2.19	0.6
Flag leaf sheath - Ear emergence	0	0.02	0.47	0.15	0.18	0.02
Roots - Three leaf stage	1.13	0.34	2.04	0.21	1.23	0.5
Axillary roots - Three leaf stage	0.95	0.37	2.86	0.39	1.56	0.47
Flag leaf sheath - 50 percent spike	0.06	0.04	0.34	0.05	0.22	0.17
Radicle - Seedling stage	0.97	0.02	2.97	0.4	1.58	0.28
Roots - 50 percent spike	0.32	0.03	2.09	1.23	1	0.27
Third leaf blade - Three leaf stage	0.16	0.23	0.29	0.35	0.16	0.12
Spikelets - 50 percent spike	1.08	0.05	2.04	0.53	1.23	0.48
Root apical meristem - Tillering stage	1.69	0.33	3.84	0.23	2.72	0.55
Grain - Ripening stage	1.03	0.56	1.09	0.17	0.97	0.57
Awns - Ear emergence	0	0.04	0.42	0.12	0.23	0.13
Glumes	0.05	0.05	0.7	0.21	0.69	0.18
Glumes - Ear emergence	0.1	0.04	1.02	0.25	0.51	0.14
Leaf ligule	0.23	0.05	1.15	0.11	0.6	0.17
Flag leaf blade - 50 percent spike	0.07	0.06	0	0.03	0.09	0.04
Internode #2 - 50 percent spike	0.19	0.13	1.28	0.67	0.57	0.06
Fifth leaf sheath - Fifth leaf stage	1.44	0.27	1.41	0.15	1.48	0.38
fifth leaf blade night (-0.25h) 21:45	0.07	0.06	0.26	0.13	0.05	0.05
Grain - Soft dough	0.66	0.28	1.8	0.2	0.67	0.17
Flag leaf blade (senescence) - Dough stage	0.16	0.05	0.5	0.08	0.11	0.05

Flag leaf blade night (-0.25h) 06:45 - Flag leaf stage	0	0.03	0.46	0.02	0	0.03
Flag leaf blade (senescence) - Ripening stage	0.24	0.04	0.44	0.05	0.21	0
First leaf blade - Tillering stage	0.05	0.05	0.45	0.15	0.12	0.04
Shoot apical meristem - Tillering stage	2.04	0.4	2.19	0.64	2.09	1.06
Shoot axis - First leaf stage	0.53	0.3	0.79	0.27	0.44	0.23
Roots - Seedling stage	1.01	0.14	3.22	0.68	1.83	0.18
Shoot axis - Milk grain stage	0.3	0.38	1.19	0.17	0.39	0.08
Fifth leaf blade - Fifth leaf stage	0.19	0.26	0.54	0.55	0.17	0.23
Flag leaf blade - Ear emergence	0	0	0.31	0.06	0.09	0.04
flag leaf blade night (+0.25h) 07:15	0.05	0.04	0.69	0.12	0.05	0.04
Fifth leaf blade night (-0.25h) 21:45	0.11	0.07	0.66	0.18	0.14	0.04
Shoot axis - Tillering stage	1.68	0.4	1.39	0.52	2.03	0.58
Stem axis - First leaf stage	0.53	0.3	0.79	0.27	0.44	0.23
Endosperm	0.48	0.05	1.07	0	0.44	0.07
Peduncle	0	0	0.56	0.19	0.23	0.11
Peduncle - 50 percent spike	0.29	0.03	1.29	0.3	0.52	0.02
Peduncle - Ear emergence	0.1	0.03	2.3	0.65	0.56	0.17
Flag leaf sheath - Full boot	0	0.01	0.28	0.04	0.13	0.13
Flag leaf blade - Flag leaf stage	0	0.01	0.39	0.33	0.13	0.05
Lemma	0.26	0.19	0.53	0.21	0.36	0.1
Lemma - Ear emergence	0.2	0.09	0.82	0.16	0.47	0.1
Awns - Milk grain stage	0	0.02	0.35	0.21	0.2	0.16
fifth leaf blade night (+0.25h) 22:15	0	0.01	0.42	0.18	0.09	0
Flag leaf blade - Milk grain stage	0.05	0.04	0.67	0.16	0.15	0.12
Grain - Hard dough	0.92	0.37	1.53	0.3	0.76	0.33
Flag leaf sheath - Milk grain stage	0.19	0.09	0.43	0.11	0.17	0.01
Embryo proper	1.78	0.19	1.4	0.32	0.71	0.33
Fifth leaf blade (senescence) - Milk grain stage	0.07	0.07	0.44	0.07	0.26	0.06
Roots - Tillering stage	1.12	0.13	3.02	0.68	1.4	0.3
Shoot axis - Full boot	1.18	0.41	2.16	0.05	1.55	0.7
Fifth leaf blade - Ear emergence	0	0.02	0.31	0.18	0	0.03
First leaf sheath - Seedling stage	0.97	0.5	1.88	0.87	0.79	0.48

Supplementary Table S2.10-F. Tissue specific expression for *Gγ4* copies

Tissue	*Gγ4-A*		*Gγ4-B*		*Gγ4-D*	
	RPKM	SD	RPKM	SD	RPKM	SD
First leaf sheath - Tillering stage	0.06	0.04	0.26	0.09	0.08	0.05
Internode #2 - Milk grain stage	0	0.01	0	0.01	0	0
Shoot apical meristem - Seedling stage	4.27	2.52	2.76	2.02	5.62	4.11
Grain - Milk grain stage	0.05	0.02	0	0.01	0.24	0.07
First leaf blade - Seedling stage	0.51	0.53	0.47	0.46	0.61	0.82
Flag leaf blade - Full boot	0	0	0.26	0.04	0.15	0.03
Awn - 50 percent spike	1.47	0.16	1.31	0.26	1.94	0.27
flag leaf blade night (-0.25h) 06:45	0	0	0	0	0	0
Shoot axis - Flag leaf stage	2.11	2.66	1.78	2.24	3.08	3.77
Fifth leaf blade - Flag leaf stage	0	0	0.23	0.06	0.15	0.09
Third leaf sheath - Three leaf stage	8.85	2.29	6.1	2.12	9.83	2.94
Internode #2 - Ear emergence	0.06	0.04	0.14	0.01	0.06	0.09
Anther	0.07	0.1	0.11	0.07	0.09	0.02
Spike	4.1	0.64	2.81	0.53	4.2	1.06
Coleoptile	2.68	0.95	1.97	0.7	2.93	0.8
Stigma and Ovary	1.86	0.96	1.93	0.11	2.73	0.93
Roots - Flag leaf stage	0.06	0.04	0.06	0	0.1	0.05
Fifth leaf sheath - Flag leaf stage	0.1	0.05	0.16	0.12	0.28	0.18
Root apical meristem - Three leaf stage	0.82	0.34	0.25	0.01	0.48	0.09
Flag leaf sheath - Ear emergence	0	0	0.05	0.02	0	0.01
Roots - Three leaf stage	4.25	5.54	3.07	3.7	4.46	5.93
Axillary roots - Three leaf stage	0.5	0.38	0.14	0.07	0.25	0.24
Flag leaf sheath - 50 percent spike	0	0.01	0	0.04	0	0.01
Radicle - Seedling stage	0.67	0.21	0.14	0.05	0.52	0.27
Roots - 50 percent spike	0.23	0.19	0	0.02	0.16	0.06
Third leaf blade - Three leaf stage	0.64	0.85	0.55	0.76	0.8	1.13
Spikelets - 50 percent spike	1.2	0.08	1.42	0.17	1.84	0.33
Root apical meristem - Tillering stage	1.36	0.2	0.47	0.13	0.6	0.09
Grain - Ripening stage	0	0	0.05	0.04	0	0.06
Awns - Ear emergence	0.28	0.11	0.22	0.09	0.6	0.14
Glumes	0	0.02	0.26	0.07	0	0.02
Glumes - Ear emergence	0.36	0.16	0.71	0.18	0.41	0.32
Leaf ligule	1.98	0.78	0.66	0.17	1.8	0.32
Flag leaf blade - 50 percent spike	0	0.01	0.11	0.04	0	0.02
Internode #2 - 50 percent spike	0	0.01	0.25	0.23	0	0.01
Fifth leaf sheath - Fifth leaf stage	12.23	2.66	9.76	2.54	14.58	3.3
fifth leaf blade night (-0.25h) 21:45	0	0	0	0.02	0	0.02
Grain - Soft dough	0.27	0.16	0.16	0.06	0.21	0.18
Flag leaf blade (senescence) - Dough stage	0	0	0	0.03	0	0

Flag leaf blade night (-0.25h) 06:45 - Flag leaf stage	0	0	0	0	0	0.01
Flag leaf blade (senescence) - Ripening stage	0	0	0	0.02	0	0
First leaf blade - Tillering stage	0	0	0.13	0.07	0	0
Shoot apical meristem - Tillering stage	2.15	2.28	1.64	1.93	2.61	2.92
Shoot axis - First leaf stage	1.16	0.9	0.99	1.27	1.34	1.2
Roots - Seedling stage	0.57	0.2	0.2	0.09	0.31	0.07
Shoot axis - Milk grain stage	0.13	0.11	0.08	0.11	0.1	0.14
Fifth leaf blade - Fifth leaf stage	0.81	1.15	0.57	0.79	1.26	1.78
Flag leaf blade - Ear emergence	0	0.01	0	0.02	0	0
flag leaf blade night (+0.25h) 07:15	0	0	0	0.01	0	0.01
Fifth leaf blade night (-0.25h) 21:45	0	0.04	0	0.01	0	0
Shoot axis - Tillering stage	1.07	1.07	0.78	0.87	1.71	1.66
Stem axis - First leaf stage	1.16	0.9	0.99	1.27	1.34	1.2
Endosperm	0.05	0.03	0.06	0.04	0.05	0.01
Peduncle	0	0.01	0.89	0.39	0.12	0.06
Peduncle - 50 percent spike	0	0.01	0	0	0	0.01
Peduncle - Ear emergence	0.11	0.05	0.06	0.04	0.19	0.16
Flag leaf sheath - Full boot	0	0.02	0.32	0.21	0.24	0.04
Flag leaf blade - Flag leaf stage	0	0	0	0.01	0	0.02
Lemma	0.1	0.03	0.39	0.1	0.08	0.02
Lemma - Ear emergence	0.48	0.04	0.53	0.27	0.14	0.08
Awns - Milk grain stage	0	0.03	0.09	0.03	0.13	0.02
fifth leaf blade night (+0.25h) 22:15	0	0	0	0.03	0	0.01
Flag leaf blade - Milk grain stage	0	0	0	0.01	0	0
Grain - Hard dough	0.23	0.09	0.15	0.1	0.28	0.18
Flag leaf sheath - Milk grain stage	0	0	0	0.04	0	0
Embryo proper	0.71	0.06	0.38	0.11	0.66	0.07
Fifth leaf blade (senescence) - Milk grain stage	0	0	0	0.02	0	0
Roots - Tillering stage	0.68	0.09	0.27	0.22	0.24	0.11
Shoot axis - Full boot	0.34	0.08	0.12	0.09	0.45	0.04
Fifth leaf blade - Ear emergence	0	0	0	0.01	0.05	0.03
First leaf sheath - Seedling stage	1.69	1.8	1.85	2.02	2.43	2.53

Supplementary Table S2.11. Tissue specific expression of G protein gene family members in *T. aestivum* thirteen tissues analysed by Affymetrix microarray

Tissue	Expression log 2 units							Fold change relative to Seedling leaf					
	GA1	***Gβ***	***Gγ1***	***Gγ2***	***Gγ3***	***Gγ4***		***GA1***	***Gβ***	***Gγ1***	***Gγ2***	***Gγ3***	***Gγ4***
Seedling, leaf	6.09	8.51	4.69	5.25	3.94	6.5		1	1	1	1	1	1
Germinating seed, coleoptile	7.33	9.44	4.6	5.19	3.99	8.43		2.38	1.91	0.93	0.96	1.03	3.82
Germinating seed, root	7.02	9.17	9.38	5.09	4.01	6.12		1.91	1.59	25.74	0.9	1.05	0.77
Germinating seed, embryo	7.35	9.39	8.48	5.08	3.99	7.28		2.4	1.85	13.83	0.89	1.04	1.71
Seedling, root	6.42	9.06	9.39	5.18	4.02	5.97		1.26	1.46	25.92	0.96	1.06	0.69
Seedling, crown	6.86	9.29	6.1	5.26	3.96	8.7		1.71	1.72	2.65	1.01	1.02	4.6
Immature inflorescence	7.15	9.8	4.83	5.04	4.12	8.39		2.09	2.46	1.1	0.87	1.13	3.72
Floral bracts, before anthesis	5.45	8.82	4.2	5.11	3.89	6.03		0.64	1.24	0.71	0.91	0.97	0.72
Pistil, before anthesis	6.36	9.25	3.95	4.12	3.8	6.29		1.21	1.68	0.6	0.46	0.91	0.86
Anthers, before anthesis	5.86	7.88	5.08	4.69	4.2	5.9		0.86	0.65	1.31	0.68	1.2	0.66
Caryopsis, 3-5 DAP	6.75	9.32	4.73	4.8	3.96	6.62		1.58	1.75	1.02	0.73	1.02	1.09
Embryo, 22 DAP	6.55	9.45	8.39	5.08	3.7	6.63		1.38	1.92	13	0.89	0.85	1.09
Endosperm, 22 DAP	5.65	8.7	8.11	5.41	4	5.39		0.74	1.15	10.67	1.12	1.05	0.47

Note: Tissue specific expression microarray data from Schreiber et al. 2009. Fold change is calculated relative to seedling leaf considering it equal to 1 and are non logarithmic.

Supplementary Table S2.12. Gene expression analysis in response to drought, heat and combined stress assayed by 61k Affymetrix microarray

Supplementary Table S2.12-A. Gene expression analysis for G protein gene families in *T. aestivum* by 61k Affymetrix microarray

	log 2 expression values for Ofanto				log 2 expression values for Cappeli			
Gene	Control	Osmotic	Heat	Combined	Control	Osmotic	Heat	Combined
GA1	2.58	2.19	2.22	2.35	2.21	2.51	2.56	2.56
Gβ	6.68	6.36	6.55	6.50	6.42	6.42	6.29	6.36
Gγ1	2.63	2.73	2.79	3.30	2.90	2.54	3.14	2.94
Gγ2	4.86	4.85	4.85	5.53	5.09	5.26	5.17	5.20
Gγ3	2.74	2.66	2.63	2.55	2.59	2.64	2.66	2.68
Gγ4	4.35	3.93	3.95	4.48	3.73	3.90	3.95	3.80

Supplementary Table S2.12-B. Fold change for G protein gene families in *T. aestivum* by 61k Affymetrix microarray

	Ofanto fold change				Cappeli fold change			
Gene	Control	Osmotic	Heat	Combined	Control	Osmotic	Heat	Combined
GA1	1	0.77	0.78	0.85	1	1.24	1.28	1.28
Gβ	1	0.80	0.91	0.88	1	1.01	0.91	0.96
Gγ1	1	1.07	1.12	1.59	1	0.78	1.18	1.03
Gγ2	1	0.99	0.99	1.59	1	1.13	1.06	1.08
Gγ3	1	0.95	0.93	0.87	1	1.03	1.05	1.06
Gγ4	1	0.75	0.76	1.09	1	1.12	1.17	1.05

Note: log2 expression in two *T. turgidum* cultivar sp. durum, Ofanto and Cappeli with low and high water use efficiency respectively were analysed from microarray data at PLEXdb. Plants were given drought, heat and combined stress at booting stage and the flag leaf tissues were used RNA isolation. Table 2.12-B denotes the non logarithmic fold change respective to value from Table 2.12-A.

Supplementary Table S2.13. Gene expression in spring and winter habit cultivars in response to cold stress assayed by microarray

Treatments	**Spring Manitou log2 expression values**						**Winter Manitou log2 expression values**					
	GA1	*Gβ*	*Gγ1*	*Gγ2*	*Gγ3*	*Gγ4*	*GA1*	*Gβ*	*Gγ1*	*Gγ2*	*Gγ3*	*Gγ4*
Control	7.03	9.36	5.91	5.07	3.85	8.03	7.18	9.41	5.97	4.87	3.71	8.02
2 days	7.87	9.24	6.32	4.97	3.83	7.83	7.74	9.33	6.29	5.05	3.87	7.79
14 days	7.39	9.31	6.25	5.05	3.68	7.95	7.41	9.34	6.24	4.88	3.76	7.83
21 days	7.32	9.24	5.91	5.06	3.86	7.88	7.49	9.18	6.43	4.99	3.79	7.54
35 days	7.54	9.27	6.02	5.05	3.93	8.05	7.51	9.32	6.04	4.99	3.91	7.88
42 days	7.05	9.26	6.54	5.05	3.71	7.60	7.24	9.31	6.52	5.05	3.77	7.65
56 days	7.16	9.24	6.47	5.30	3.74	7.29	7.22	9.39	6.32	5.17	3.84	7.78
70 days	6.99	9.18	7.17	5.32	3.74	6.26	7.03	9.34	6.80	5.25	3.54	7.23
DACS	**Fold change spring Manitou**						**Fold change winter Manitou**					
Control	1	1	1	1	1	1	1	1	1	1	1	1
2 days	1.78	0.92	1.33	0.93	0.99	0.87	1.47	0.95	1.25	1.13	1.11	0.85
14 days	1.28	0.97	1.27	0.98	0.89	0.95	1.18	0.95	1.21	1.01	1.03	0.88
21 days	1.22	0.92	1.00	0.99	1.01	0.90	1.24	0.85	1.38	1.09	1.06	0.72
35 days	1.42	0.94	1.08	0.99	1.06	1.01	1.26	0.94	1.05	1.09	1.14	0.91
42 days	1.01	0.94	1.55	0.99	0.91	0.74	1.05	0.94	1.47	1.13	1.04	0.78
56 days	1.09	0.92	1.48	1.17	0.93	0.60	1.03	0.99	1.27	1.23	1.09	0.85
70 days	0.97	0.89	2.39	1.19	0.93	0.29	0.90	0.95	1.78	1.30	0.89	0.58
Treatments	**Spring Norstar log 2 expression values**						**Winter Norstar log 2 expression values**					
Control	7.07	9.40	5.66	5.21	4.28	8.22	7.15	9.38	5.82	5.13	4.29	8.31
2 days	7.63	9.26	6.05	5.24	4.18	7.51	7.65	9.23	6.02	5.27	4.38	7.29
14 days	7.32	9.28	6.68	5.13	4.24	7.95	7.11	9.21	6.79	5.20	4.28	7.49
21 days	7.48	9.15	6.49	5.28	4.23	7.60	7.33	9.07	6.48	5.25	4.19	7.09
35 days	7.42	9.25	6.22	5.26	4.30	8.25	7.34	9.22	6.20	5.35	4.15	7.23
42 days	7.05	9.18	6.93	5.53	4.13	7.72	7.23	9.31	6.99	5.24	4.22	7.26
56 days	7.00	9.27	6.71	5.14	4.13	7.76	7.03	9.19	6.99	5.22	4.12	7.08
70 days	6.91	9.24	7.31	5.29	4.08	6.94	7.15	9.28	6.94	5.20	4.16	7.59
DACS	**Fold change spring Norstar**						**Fold change winter Norstar**					
Control	1	1	1	1	1	1	1	1	1	1	1	1
2 days	1.48	0.91	1.31	1.02	0.94	0.61	1.41	0.90	1.15	1.10	1.06	0.50
14 days	1.20	0.92	2.04	0.94	0.98	0.83	0.98	0.89	1.96	1.05	0.99	0.57
21 days	1.34	0.84	1.78	1.05	0.97	0.65	1.14	0.80	1.58	1.08	0.93	0.43
35 days	1.28	0.90	1.47	1.04	1.02	1.02	1.15	0.90	1.30	1.16	0.91	0.47
42 days	0.99	0.86	2.42	1.25	0.90	0.71	1.06	0.95	2.25	1.08	0.96	0.48
56 days	0.96	0.92	2.08	0.95	0.90	0.73	0.92	0.87	2.24	1.06	0.89	0.43
70 days	0.90	0.89	3.13	1.06	0.87	0.41	1.00	0.93	2.17	1.05	0.91	0.61

Note: Expression values are given in log2 units. DACS- Days after cold stress. Fold changes calculated are non logarithmic.

Chapter 3: Brachypodium CLO7 interact with G protein α subunit and modulate lateral root growth under osmotic stress

3.1. Abstract

Very few regulators of heterotrimeric G proteins in plants have been identified and shown to modulate the plant growth and developmental processes under environmental stress conditions but the knowledge about the proteins that interact with G proteins in regulation of root growth under ABA and osmotic stress require study. Caleosins, calcium binding proteins with a single EF hand, are one of such class of proteins; they have been reported to interact with the heterotrimeric G protein α subunits in *Triticum aestivum* and *Arabidopsis thaliana*. Some members of the gene family are known to be transcriptionally induced by abiotic stress and hormonal treatments and to regulate stomatal aperture under ABA inducing stress conditions. Here we have reported the physical interaction of *Brachypodium distachyon* Caleosin 7 (Bd-CLO7) with the Gα subunit (Bd-Gα) of the heterotrimeric G protein complex assayed by bimolecular fluorescence complementation. Intracellular localization using the fusion with the full length GFP showed that Bd-Gα was localized to plasma membrane and endoplasmic reticulum, and Bd-CLO7 was localized to ER, whereas the interaction between these two proteins was localized to plasma membrane. The Bd-*clo7* mutant had 12% longer primary root lengths than WT in control conditions and *CLO7* plays role in reduction of PR length in response to mannitol. Bd-CLO7 does not significantly affect coleoptile node root growth under ABA or osmotic stress conditions but plays a role in osmotic stress induced repression of lateral root development. Bd-*clo7* mutant was less sensitive to lateral root growth reduction in response to 150 mM mannitol than the wild type Bd21-3. Bd21-3 showed 42% and 54% reductions in total lateral root numbers and lengths respectively, whereas Bd-*clo7* mutant showed 5% and 15% reduction for lateral root number and lateral root lengths, respectively, for the same treatment. Though ABA treatment decreases lateral root development, the WT and mutant did not show any significant differences for lateral root growth in response to ABA. This study showed that Bd-*CLO7* negatively regulates the lateral root growth under osmotic stress through ABA independent signaling pathway.

3.2. Introduction

Drought, salinity and extreme temperatures are abiotic stress conditions that affect crops

worldwide causing reductions in the crop yield to the extent of 50%, depending upon the severity of the stress and the growth stage at which the plant is affected (Rodziewicz et al., 2014). Abiotic stress conditions including drought and salinity play a major role in affecting the plant growth and crop productivity by reducing photosynthetic rates, and causing wilting followed by programmed cell death, which are regulated at molecular level by plant hormones including abscisic acid (ABA), and by signalling molecules including protein kinases and phosphatases, ion transporters and transcription factors (Landi et al., 2017). The hormone ABA plays a key role in abiotic stress responses. The adaptive responses observed in plants such as stomatal closure and alteration in root architecture are regulated by ABA, however the comparison of induction of drought stress responsive and genes induction caused by exogenous ABA application showed that some of the drought stress responsive genes are ABA dependent, whereas others are ABA independent (Sah et al., 2016; Shinozaki and Yamaguchi-Shinozaki, 2006).

The different abiotic stress conditions including cold, heat, salt, drought, osmotic stress and mechanical stress increase the concentration of the abscisic acid in the plant, which is also associated with the elevated level of the cytoplasmic calcium levels that acts as a secondary messenger known as calcium signatures (Edel and Kudla, 2016; Knight et al., 1996). Calcium binding proteins respond to increased calcium levels and further regulate the signalling cascade associated with the physiological responses in the plants (Reddy et al., 2011). Calcium binding proteins including calmodulins (CMs), calmodulin-like (CML) proteins and calcineurin B-like (CBL's) proteins have one to six conserved calcium binding domains called EF hands. Caleosins are such calcium binding proteins, each with a single conserved calcium binding EF hand and are known to play important roles in stress responses including ABA signaling, drought and salinity responses (Kim et al., 2011; Aubert et al., 2010). Arabidopsis caleosin *CLO3,* also known as Response to Dehydration 20 (*RD20*) and At-*CLO4* act as negative regulators of ABA signalling in seed germination and drought tolerance. At-*CLO3* is known to be induced by the ABA, salt and drought and has been reported to play a role in the control of stomatal aperture and in drought tolerance (Kim et al., 2011; Aubert et al., 2010).

Members of the caleosin gene families in Arabidopsis, At-*CLO3* and At-*CLO7* had been shown to interact with the heterotrimeric G protein alpha subunit (Gα), GPA1, in the Gulick lab (Wang, Z., M.Sc. thesis, 2009 and unpublished). The Gα subunits in different plant species have been

shown to interact with other proteins and to regulate plant physiological processes and hormone signalling pathways. For example, the Arabidopsis Gα protein (GPA1) is known to interact with plastid protein thylakoid formation1 (THF1), G-protein coupled receptor 1 (GCR1) and Phospholipase Dα1 (PLDα1) (Huang et al., 2006; Pandey and Assmann, 2004; Zhao and Wang, 2004). The rice Gα protein, RGA1 and the wheat Gα, Ta*Gα*-7A have been shown to interact with protein COLD1, which encodes a G protein coupled receptor like protein, and to confer cold tolerance in japonica rice through calcium mediated signaling pathway. The gene encoding the Gα subunit was also shown to regulate plant height in wheat cultivar Kenong 199 (Ma et al., 2015; Dong et al., 2019). The caleosin in *Triticum aestivum*, Ta-*Clo3* has been reported to interact with the Gα protein, GA3, by *in vivo* and *in vitro* protein interaction assays (Khalil et al., 2011). This suggests that the Gα protein has several interacting protein partners which are conserved in different species and the members of caleosin gene families are one class of such proteins. Moreover both GPA1 and caleosins in Arabidopsis are known to be involved in ABA and stress mediated responses. Hence it is better to study the GPA1 and caleosins in conjugation.

Brachypodium distachyon is a diploid model monocot plant species used extensively for studying the functional genomics in the grasses, including the cereals, due to its small genome size, simple growing habits and short life cycle. Brachypodium is closely related to the wheat and the root growth characters are similar for both these species except wheat has complex root system. The Brachypodium diploid genome is also less complex than the hexaploid genome of *T. aestivum*. The adaptive responses to stress conditions should be similar in the cereals and Brachypodium. However, the easy availability of the mutants in Brachypodium makes is an advantage to study the function of different genes involved in stress responses (Draper et al., 2001). Genes encoding ten caleosins have been reported in *B. distachyon* and the expression analysis using RNA-Seq and microarray showed that *CALEOSIN7 (CLO7)* in Brachypodium is induced by abiotic stress conditions which include cold, drought and salinity stress (Hao et al., 2017; Verelst et al., 2013; Guo et al., 2020), whereas the transcriptional downregulation for the same gene has been detected under submergence stress (Rivera-Contreras et al., 2016). The interacting proteins and the role they play in plant growth and in the plant's response to environmental stresses are not well studied in Brachypodium (Khalil et al., 2014). The mutant analysis of the members of caleosin gene families is an important approach in characterizing the possible role of caleosins in ABA and osmotic stress signaling in *B. distachyon*. Here, we report the *in vivo* interaction of

Brachypodium *CALEOSIN7* (Bd-*CLO7*) with G protein alpha subunit (Bd-*Gα)* using bimolecular fluorescence complementation and the characterization of the effect of ABA and osmotic stress on the root growth of Bd-*clo7* mutant.

3.3. Material and Methods

3.3.1. Plant Material and Growth conditions

Seeds for Bd-*clo7* mutant (T-DNA insertion JJ9005 line with insertion in the exonic region) were received from DOE Joint Genome Institute, (Walnut Creek, California). Plants were grown in 3:1:1 mixture of black soil: peat moss: vermiculite in a greenhouse under long day conditions at 21-24°C. Natural light was supplemented with artificial lighting to achieve 16 hrs of illumination. The homozygous mutant lines were identified in the T3 generation by screening by PCR using primers listed in **Table 3.1.**

Table 3.1. Primers used for screening *Brachypodium distachyon clo7* mutant

Specific primers	Primers name	Primer sequence
For *CLO7* gene	Bd-*CLO7*FP gene	ATGGCTCTGTTGCACGCT
	Bd-*CLO7*RP gene	CACCTTCGTAGGTCTCGGAGG
For T-DNA	Bd-*CLO7*FP2 gene	GAGAGAGAGAGAGAAGAAGAAGAAG
	RP T3 T-DNA LB	AGCTGTTTCCTGTGTGAAATTG

Seeds for WT and mutants were cold stratified in wet paper towels for ten days at 4°C. The seed paleas were removed and seeds were sterilized using 2% sodium hypochlorite (diluted from 10.3% household bleach) 0.001% of Triton X-100 for 10 minutes, followed by 4-5 washes of sterilized distilled water. Seed were germinated on sterile MS media plates (0.2% MS salt, 0.05% MES hydrate, 2% sucrose, adjusted to pH 5.7 with by addition of KOH). Three days post-germination, seedlings were transferred to treatment plates containing MS media supplemented with ABA at concentrations of 2 µM, 1 µM, 0.5 µM, 0.05 µM and 0.025 µM, and MS plates supplemented with mannitol at 300 mM, 150 mM, 100 mM, 50 mM and 25 mM. The primary root growth was highly sensitive to the 2 µM ABA and 300 mM mannitol concentrations which resulted in almost complete arrest of growth in the WT and *clo7* mutant. To create moderate stress where the primary root growth reduction was nearly 30-50% of control conditions, 0.05 µM of ABA and 150 mM of mannitol concentrations were used for further characterization. The

seedlings were grown in an E15 Conviron growth chamber under long day light conditions (16hrs light and 8hrs dark) at 22°C and light intensity of 100-120 μE m^{-2} s^{-1}.

3.3.2. Intracellular localization and bimolecular fluorescence complementation

To investigate the subcellular localization and *in vivo* interaction of Bd-*Gα* and Bd-*CLO7*, the full-length cDNAs for Bd-*Gα* (Bradi2g60350.1) and Bd-*CLO7* (Bradi1g44200) were synthesized with codon bias adjusted for the expression in *Nicotiana benthamiana* by GeneArt® Strings™ DNA Fragments (Carlsbad, California, United States). The sequences are given in **Supplementary Table S3.1.** Coding regions without stop codon were amplified using primers with Gateway ends. The full-length coding regions of Bd-*Gα* and Bd-*CLO7* were cloned into pDONR207 entry vectors using BP reactions, and were subsequently subcloned by *in vitro* recombination into Gateway C-terminal GFP tag plant expression vector PK7FWG2 using LR reactions. Bd-*Gα* and Bd-*CLO7* were also subcloned into Bimolecular Fluorescence Complementation (BiFC) vectors with C and N terminal half YFP tags, pBatL-B-sYFP-N and pBatL-B-sYFP-C respectively, using Gateway LR reactions. The details for primers and the vectors used to make constructs are given in **Table 3.2 and 3.3.**

Table 3.2. Primers used in cloning Brachypodium *Gα* and *CLO7*

Gene name	Primer name	Primer sequence
Bd-Gα	Bd-*Gα* FP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGCT CATCTTGCTCTAG
	Bd-*Gα* RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGTCTCTTC CCTAGATCTCCG
Bd-*CLO7*	Bd-*CLO7* FP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTAG CAAGTCTGCTAACAC
	Bd-*CLO7* RP	GGGGACCACTTTGTACAAGAAAGCTGGGTACTTCTTCTT G GCGGACTCTTTA

Table 3.3. Vectors used in Gateway cloning of plasmid constructs for BiFC assay

Vector name	Antibiotic resistance	Resistance for antibiotic
pDONR 207	Kanamycin	BP cloning entry vector
pK7FWG2	Spectinomycin	LR cloing C-terminal GFP tag vector
pBatL-B-sYFP-N	Spectinomycin	LR cloning N-terminal YFP tag
pBatL-B-sYFP-C	Spectinomycin	LR cloning C-terminal YFP tag

The GFP or YFP expression plasmid constructs were transformed into electrocompetent *Agrobacterium tumefaciens* strain AGL1. A vector for expression of the p19 protein of tomato bushy stunt virus (TBSV) under the regulation of the 35S promoter which is known to supress the gene silencing during gene expression in plants was also transformed with the experimental constructs. The cultures for the strains containing the clones Bd-*Gα*-nYFP, Bd-*CLO7*-cYFP, p19, and markers for the plasma membrane (PM) and endoplasmic reticulum (ER) fused with the mCherry fluorescent tag were grown overnight at 30°C (Nelson et al., 2007). Cultures were centrifuged and the pellets were resuspended to specific ODs. Cultures with the GFP and BiFC constructs, and p19 were suspended at OD600 of 0.5. The PM or ER markers were resuspended at OD600 of 0.01 or 0.02. For BiFC, cultures carrying Bd-*Gα*-n-YFP, Bd-*CLO7*-c-YFP and p19 and the PM or ER markers were mixed in equal volumes to final volume of 3 ml. Empty pBatL-B-sYFP-N or pBatL-B-sYFP-C in combination with Bd-*CLO7*-C-YFP or Bd-*Gα*-N-YFP respectively were used as negative controls. The mixtures were centrifuged at 4000g for 20 min and the pellet obtained was suspended in 3 ml of Agroinfiltration solution containing 10 mM $MgCl_2$ and 150 μM acetosyringone made with sterilized distilled water. The cultures were incubated at room temperature for 4 hrs and were used to infiltrate 4-5 week old *N. benthamiana* plants on the abaxial leaf surface with a syringe. The plants were kept at 21-24°C under long day conditions in greenhouse and were imaged after approximately 40 hrs for intracellular localization and after 53 hrs for BiFC by Olympus Fluoview FV10i confocal laser scanning microscope at Centre for Microscopy and Cellular Imaging at Concordia University. The wavelengths of 489 nm and 480 nm were used to excite GFP and YFP by laser diode, and images were collected through 510 nm and 527 nm filters respectively. The mCherry red fluorescent protein for the PM and ER markers were excited at wavelength of 559 nm and images were collected through a 570-620 nm filter.

3.3.3. Root growth measurements

To determine the effect of ABA and osmotic stress on the root growth in Brachypodium *clo7* mutant and WT, the roots were imaged on days 9, 12 or 14, after the transfer of three day old seedlings to treatment plates or to control plates. The digital images were captured using a Nikon digital camera and root growth was quantified using Image J version 1.43 software. The plants

were scored for the primary root length, coleoptile node root number and lengths, and lateral root number and lengths.

3.3.4. Statistical analysis

Two-way ANOVA was carried out to characterize the root phenotypic response of WT and Bd-*clo7* mutant genotypes to 0.05 µM ABA or 150 mM mannitol treatment. GxT interaction effects were assayed to determine if the genotypes responded differently to the treatments. Statistical difference for root growth parameters measured in the two genotypes were analysed using both two-way and one-way ANOVA followed by Duncan's Multiple Range test. Mann-Whitney rank sum test was used to determine the statistical differences between genotypes and treatments for CNR numbers and lengths in response to ABA or osmotic stress treatment.

3.4. Results

The uniform germination of the seeds can be a crucial step in the phenotypic analysis for root growth in plant. For *B. distachyo*n, we found that seven to ten days cold stratification can enhance synchronous seed germination which results in seedlings with the uniform primary root lengths. This is advantageous in determining the root phenotypes. We used cold stratification for period of the ten days for all root phenotype experiments. The aim of this study was to check if the *CLO7* has role in the regulation of the root growth. The comparison of the difference in the root growth phenotype of *clo7* mutant and WT under ABA or mannitol treatment will indicate if the regulation of root growth by *CLO7* is mediated through ABA dependent or independent pathway.

3.4.1. Bd-*clo7* mutant had more reduction in primary root growth than WT in response to ABA

Bd-*clo7* mutant showed 12% longer primary root compared to wild type under control conditions. The Bd-*clo7* mutant had a significantly greater reduction of primary root growth under ABA treatment. In response to 0.05 µM ABA, the Bd-*clo7* mutant showed 50% primary root growth reduction, whereas wild type Bd21-3 had 42% reduction for the same treatment. In response to 150 mM mannitol treatment the Bd-*clo7* mutant and WT showed the 47% and 46% reduction in this treatment. These results suggest that *CLO7* in Brachypodium is a negative regulator of PR growth under control conditions and causes less reduction in PR length in

response to ABA, this is to say, it is a negative regulator of the inhibitory response to ABA, whereas under osmotic stress *CLO7* supresses PR growth. Two way ANOVA indicated significant genotype by treatment interaction effects which indicate that the two genotypes responded differently to the treatment. The effect of ABA and mannitol for the primary root growth is shown in **Figure 3.1** and results for two-way ANOVA is given in **Table 3.4.**

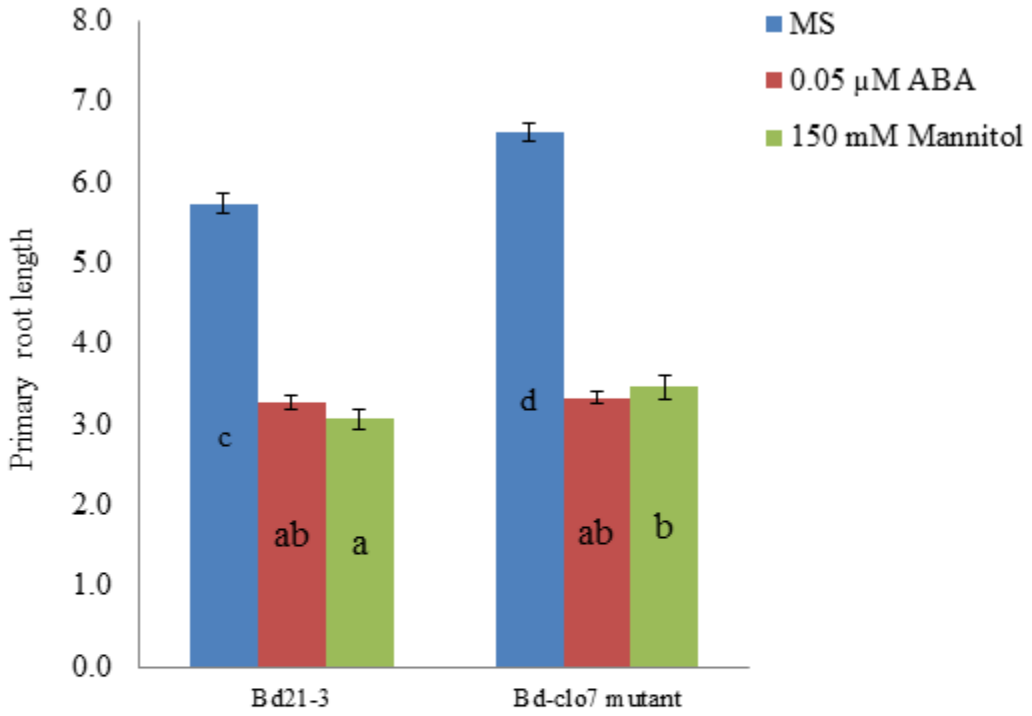

Figure 3.1. The effect of ABA and mannitol on the primary root growth of wild type Bd21-3 and Bd-*clo7* mutant. Primary root lengths in wild type, Bd21-3 and Bd-*clo7* mutant under control conditions, ABA and mannitol treatments was measured nine days after transfer of three days old seedlings to the MS control plates or plates with MS supplemented with 0.05 µM ABA or 150 mM mannitol. The significant differences were analysed by one way ANOVA ($p< 0.05$) followed by Duncan's multiple range test. The letters on the bars indicate the ranks assigned by Duncans multiple range test; bars that do not include the same letter are significantly different. Error bars represent standard errors of means.

Table 3.4. Two way ANOVA for effect of 0.05 µM ABA or 150 mM mannitol on primary root growth

Bd21-3 compared to Bd-*clo7*	p-value	
	Primary root length	
	0.05 µM ABA	150 mM Mannitol
Genotype	0.000	0.000
Treatment	0.000	0.000
Genotype × Treatment	0.000	0.050

Note: The effect of 0.05 µM ABA or 150 mM mannitol on primary root growth of WT Bd21-3 and Bd-*clo7* was determined on day 9 after the treatment. Values represent the p-values.

3.4.2. Coleoptile node root growth under ABA and osmotic stress

Brachypodium distachyon generally develops two postembryonic coleoptile node roots (CNR) and can have up to three CNR in some cases. As the name implies, these are roots that emerge from the coleoptile, a protective sheath that encloses the emerging shoot in monocotyledonous plants. The root growth characterization on day 12 after transfer of three days old seedling to control conditions, did not show any CNR growth for wild type and Bd-*clo7* mutant, whereas in both the WT and the Bd-*clo7* mutant the 0.05 µM ABA treatment induced CNR (**Figure 3.2**). The WT had 21% more CNRs (WT: 1.9±0.1; Bd-*clo7* mutant: 1.5±0.3) and 29% greater total CNR lengths than the Bd-*clo7* mutant, (WT: 3.4±0.3; Bd-*clo7* mutant: 2.4±0.7) however these differences were not statistically significant. This suggests that Bd-*CLO7* does not affect coleoptile node root growth under ABA stress. The details for the effect of 0.05 µM ABA on CNR growth is shown in **Figure 3.3** and the results for Mann-Whitney rank sum test is given in **Table 3.5.**

The WT did not show any CNR growth in control condition nor were CNR developed in response to 150 mM mannitol treatments by day 12. Bd-*clo7* mutant did not show CNR growth under control condition but had a small number of CNR in response to 150 mM mannitol treatment **(Figure 3.3)**. One in four Bd-*clo7* plants developed CNRs by day 12 of the mannitol treatment. The results for Mann-Whitney rank sum test for effect of mannitol on CNR growth did not indicate that the differences between the genotypes were statistically significant; they are summarized in **Table 3.5**. Altogether these results suggest that Brachypodium *CLO7* does not

affect coleoptile node root growth in control conditions nor in response to ABA but does have a small effect on CNR root development in response to mannitol.

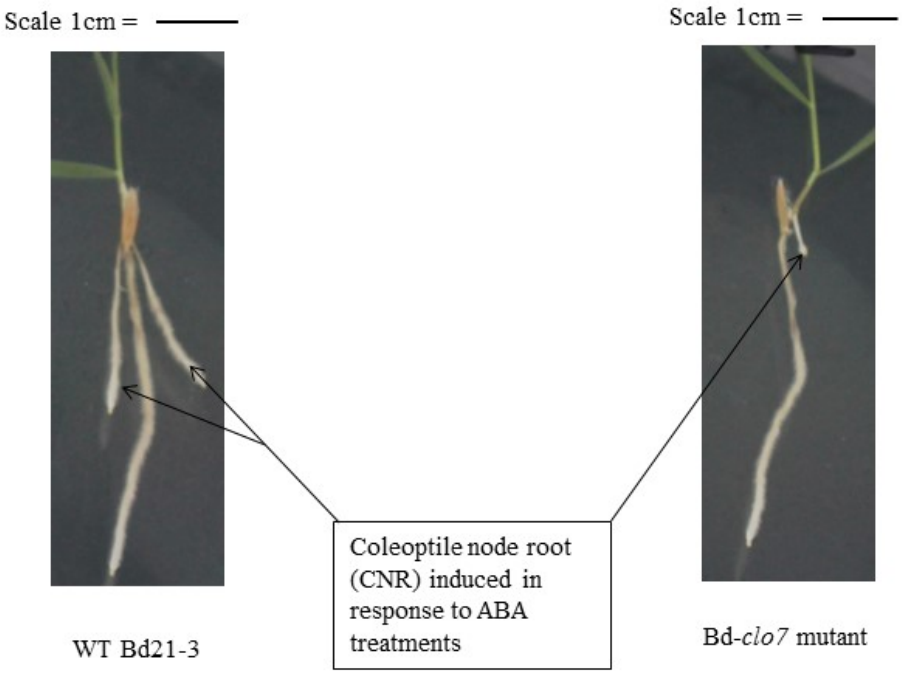

Figure 3.2. Coleoptile node roots (CNR) induced by ABA stress. The coleoptile node roots for WT Bd21-3 and Bd-*clo7* mutants were measured on day 12, after the transfer of three days old seedling to media supplemented with 0.05 µM ABA.

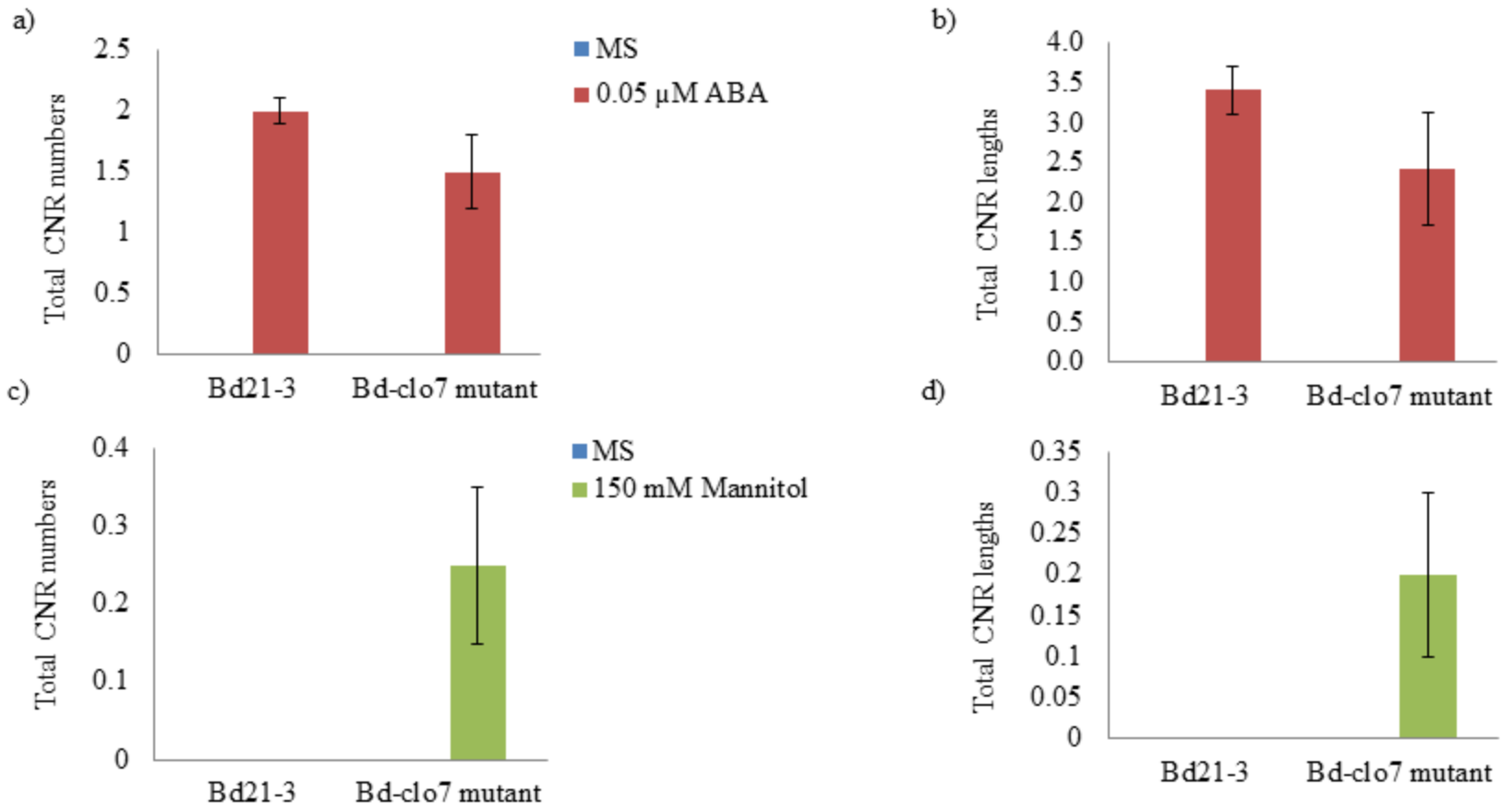

Figure 3.3. The effect of ABA or mannitol on coleoptile node root (CNR) growth in WT and Bd-*clo7* mutant plants. The a) total number of CNR, b) total length of CNR in response to 0.05 µM ABA, c) total number of CNR d) total lengths of CNR in response to 150 mM mannitol in wild type and Bd-*clo7* mutant were measured after 12 days of growth on media supplemented with of 0.05 µM ABA or 150 mM mannitol and on MS control plates. The differences were analysed by Mann-Whitney rank sum test ($p \leq 0.05$). CNR numbers or lengths between WT Bd21-3 and Bd-*clo7* mutant did not differ significantly in control, ABA or mannitol treatments. Error bars represent standard errors of means.

Table 3.5. Mann-Whitney rank sum test for the effect of ABA and mannitol on coleoptile node root growth

Bd21-3 compared to *Bd-clo7*	p-value	
	Total coleoptile root number	Total coleoptile root length
Control treatment	1.000	1.000
ABA treatment	0.393	0.247
Mannitol treatment	0.319	0.319

Note: The effect of 0.05 µM ABA or 150 mM mannitol on total coleoptile root numbers and lengths of WT Bd21-3 and Bd-*clo7* mutant was determined day 12 after the treatment. Mann-Whitney rank sum test was used to determine the significant differences between WT and Bd-*clo7* mutant in control, ABA and mannitol treatments. Values represent the p-values.

3.4.3. Bd-*CLO7* affects lateral root growth in response to osmotic stress and not ABA

The WT and Bd-*clo7* mutant had dramatically different responses to osmotic stress administered by mannitol addition to the media but had similar degrees of suppression of lateral roots development in response to ABA treatment. The effect of the osmotic stress on lateral root growth reduction was analysed after 14 days of the 150 mM mannitol treatments. Bd-*clo7* mutant showed more lateral roots and greater total lateral root lengths than wild type under osmotic stress conditions. The wild type Bd21-3 had a significantly greater reduction in the lateral root growth in mannitol treatments than the Bd-*clo7* mutant (**Table 3.6**). The total lateral root numbers and lateral root lengths in WT were reduced by 43% and 53% whereas the Bd-*clo7* mutant only showed the reduction of 5% and 14% in total lateral root numbers and lengths respectively (**Figure 3.4)** indicating that *CLO7* acts as negative regulator of lateral root development under osmotic stress conditions. Lateral root growth measured on day nine showed that Bd-*clo7* mutant had 18% more lateral roots and 17% greater total lateral root lengths than wild type under control conditions. The WT grown with of 0.05 µM ABA treatment had 61% reduction in total lateral root numbers, whereas the *clo7* mutant had a 62% reduction in lateral roots for the same conditions. Similarly, the WT had a 55% reduction in total lateral root length, whereas the *clo7* mutant had a 50% reduction (**Figure 3.4**) and (**Table 3.7**). Taken together, this indicates that Bd-*CLO7* is involved in suppression of lateral root growth under osmotic stress through ABA independent regulation.

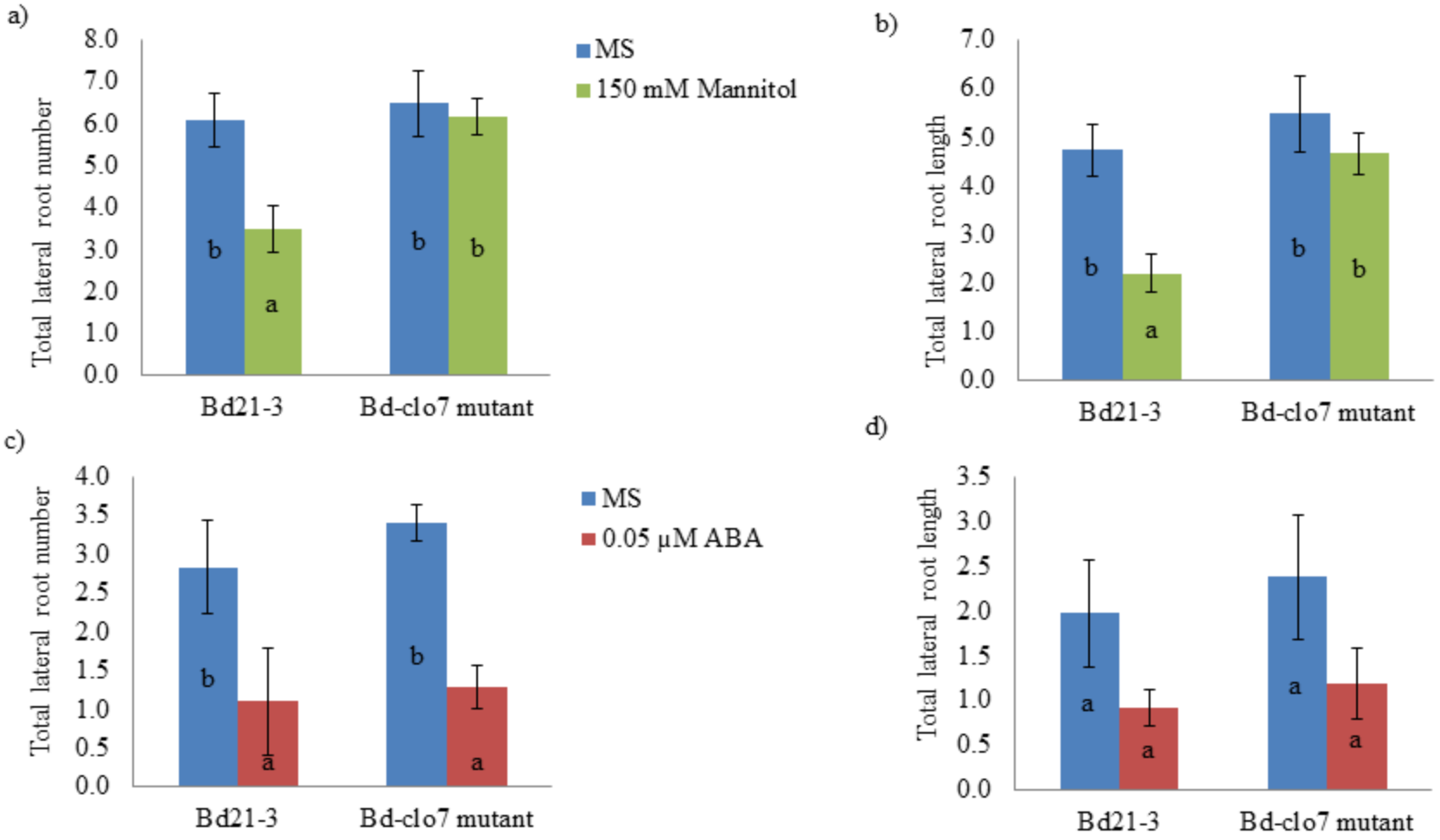

Figure 3.4. The effect of mannitol and ABA on the reduction of lateral root growth in WT and Bd-*clo7* mutant. The effect of 150 mM mannitol on reduction of a) Total number of lateral roots and b) Total lateral root length was measured on 14 days, after the transfer of the three days old seedlings to treatment media. The effect of 0.05 µM ABA on reduction of c) Total number of lateral roots and d) Total lateral root length was measured after nine days of growth on treatment media. The letters on bars indicates the ranks assigned by Duncan's Multiple Range test and error bars represent standard errors of the means.

Table 3.6. Two way ANOVA for effect of 150 mM mannitol on total lateral root growth

Bd21-3 compared to Bd-*clo7*	p-value	
	Total lateral root numbers	Total lateral root length
Genotype	0.013	0.03
Treatment	0.032	0.02
Genotype × Treatment	0.022	0.019

Note: The effect of 150 mM mannitol on total lateral root numbers and lengths of WT Bd21-3 and Bd-*clo7* mutant was determined on day 14 after the treatment. The statistical analysis was performed on log transformed data. Values represent the p-values.

Table 3.7. Two way ANOVA for effect of 0.05 µM ABA on total lateral root growth

Bd21-3 compared to Bd-*clo7*	p-value	
	Total lateral root numbers	Total lateral root length
Genotype	0.531	0.498
Treatment	0.001	0.028
Genotype × Treatment	0.644	0.885

Note: The effect of 0.05 µM ABA on total lateral root numbers and lengths of WT Bd21-3 and Bd-*clo7* mutant was determined on day 9 after the treatment. Values represent the p-values.

3.4.4. Brachypodium Gα interact with CLO7

The intracellular localization studied in the leaf epidermal tissue of 4-6 week old *N. benthamiana* plants showed that Brachypodium Gα was localized to the plasma membrane and endoplasmic reticulum, whereas Bd-CLO7 was localized to the endoplasmic reticulum. This was shown by the overlap of the signal with the ER marker and by continuous network structure of ER (**Figure 3.5).** Brachypodium Gα and CLO7 were shown to interact *in vivo* by BiFC and the interaction for these proteins was found to be localized to the plasma membrane shown by overlap with the mCherry plasma membrane marker. The specificity of the protein interaction of Bd- Gα and Bd-CLO7 was confirmed with negative controls. The negative controls of pBatL-B-sYFP-N or pBatL-B-sYFP-C without fusion proteins did not show interaction with Bd-*CLO7*-C-YFP or Bd-*Gα*-N-YFP respectively**, Figure 3.6.**

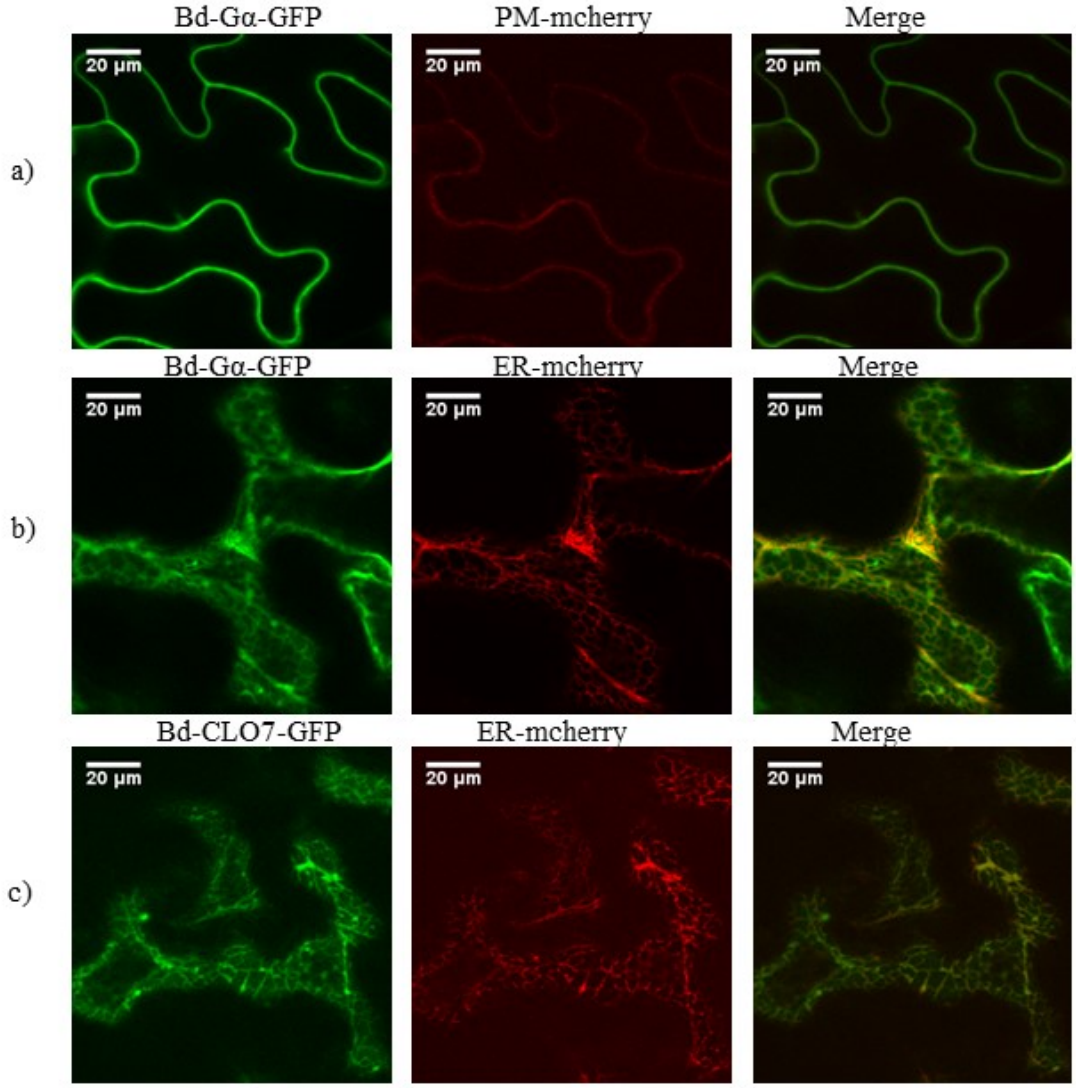

Figure 3.5. Intercellular localization of Bd-Gα-GFP and Bd-CLO7-GFP by transient expression in *N. benthamiana* epidermal leaf pavement cells. a) Bd-Gα-GFP with a plasma membrane marker and b) Bd-Gα-GFP with the endoplasmic reticulum marker and c) Bd-CLO7-GFP with the endoplasmic reticulum marker. The genes are cloned in C-terminal GFP vector PK7FWG2 and the intercellular localization is studied in the epidermal tissue of 4-6 week old *N. benthamiana*. Merge images indicate the overlap of the GFP with markers; Scale bar = 20 µm. The PM and ER markers are captured at different focal planes; hence, they are shown in different panels.

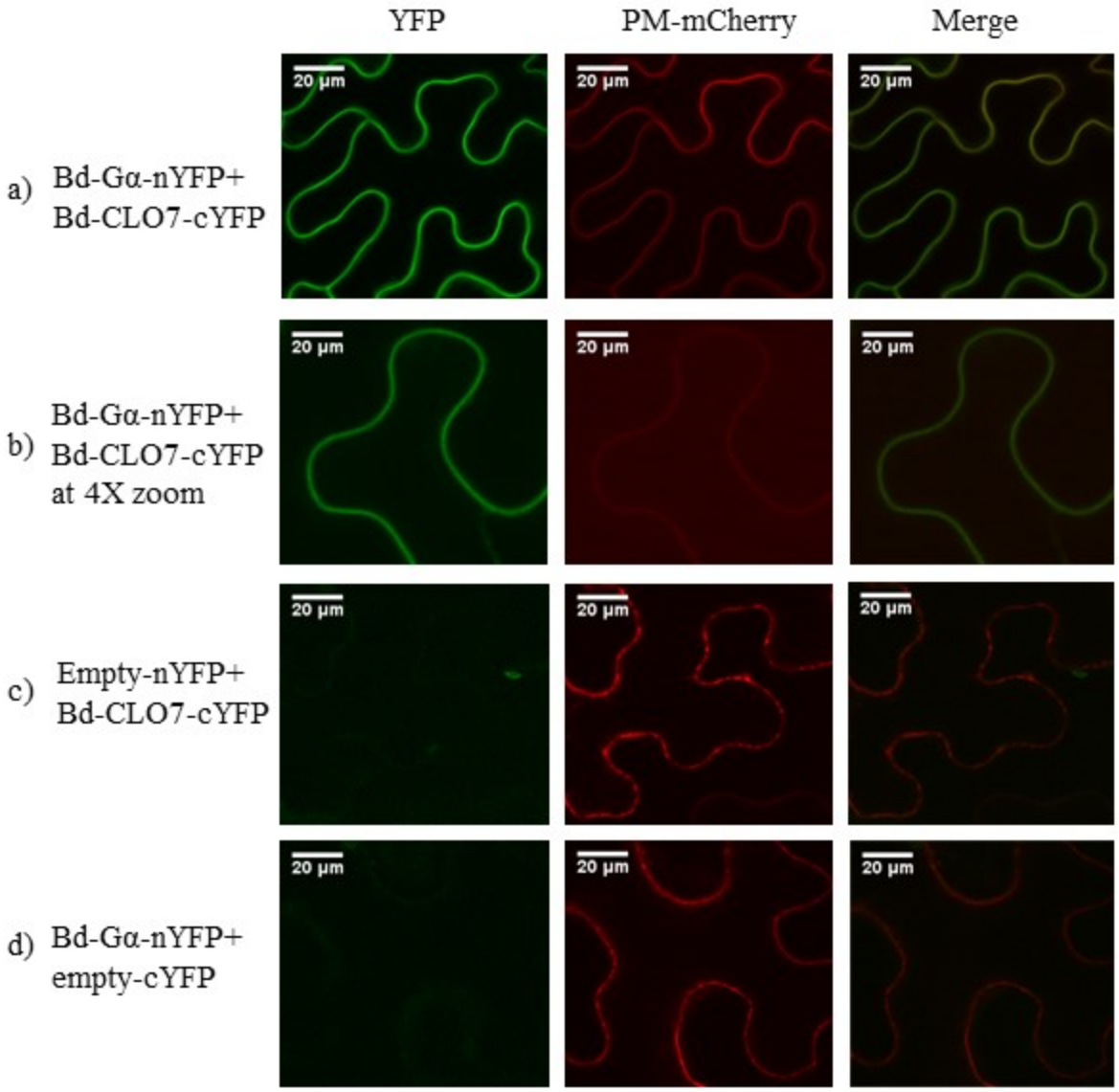

Figure 3.6. *In vivo* protein-protein interaction of *B. distachyon* Gα and CLO7 analyzed in 4-6 week old *N. benthamiana* plant epidermal leaf pavement cells by BiFC. a) Protein- protein interaction of *B. distachyon* Gα and CLO7 with plasma membrane marker (PM) b) interaction at 4X zoom, c) Empty-nYFP+Bd-CLO7-cYFP and d) Bd-Gα-nYFP+empty-cYFP are the negative controls where N and C terminal YFP tag were empty construct. The genes Bd-*Gα* and Bd-*CLO7* are cloned in N- and C-terminals of YFP vector pBatL-B-sYFP respectively. Merge image indicate the overlap of YFP with marker; Scale bar = 20 µM.

3.5. Discussion

3.5.1. Bd-Gα and Bd-CLO7 protein-protein interaction

The protein-protein interaction between Bd-Gα and Bd-CLO7 was localized to the plasma membrane whereas Bd-Gα was localized individually as a fusion to full length GFP to the plasma membrane and endoplasmic reticulum, and Bd-CLO7 was individually localized to the endoplasmic reticulum. The individual localization of Bd-Gα to the PM and ER suggest the detectable amount of Bd-Gα in these two organelles, whereas the localization of individual CLO7 to ER and the localization of Bd-Gα and Bd-CLO7 interaction to the PM suggest the movement of CLO7 to the PM or the presence of CLO7 in the plasma membrane at low levels which could not be detected by the GFP fusion. The protein-protein interaction for proteins encoded by other of the caleosin gene family members and G protein α subunits have been reported, which include the interactions for Ta-GA3 and Ta-Clo3 from *T. aestivum*, GPA and CLO3, and GPA1 and CLO7 in Arabidopsis (Khalil et al., 2011; Wang Z., 2009 thesis, and unpublished). Similar localization to the plasma membrane for the interaction between Ta-GA1, previously referred to as GA3, and Ta-Clo3 from *T. aestivum* had been reported by Khalil et al. 2011. These interactions suggest that members of the caleosins gene family and the heterotrimeric G proteins may act in the same signalling pathways.

Previous studies in Arabidopsis and wheat had shown that Gα subunits in these plants interact with the Caleosins (Khalil et al., 2011; Wang, 2009) and the interaction between CLO3 and GA1 in wheat was enhanced by Calcium Ca^{2+} (Khalil et al., 2011). At a higher level of Ca^{2+} binding of CLO3 to GA1 was not different if it was in a GTP or GDP bound state. This indicates that elevated Ca^{2+} levels possibly recruits the caleosins which interact with Gα and regulate the adaptive responses to cope with stress conditions. However, it will be interesting to know the effect of the increasing Ca^{2+} on the Bd-Gα and Bd-CLO7 interaction in Brachypodium.

The *gpa1* mutant in Arabidopsis was hypersensitive to the root growth in response to ABA treatment (Chen et al., 2006) and had increased ABA sensitivity and transpiration efficiency (Nilson and Assman, 2010), whereas *clo7* mutant does not respond differently to ABA treatment. The *gpa1* mutant in Arabidopsis had normal primary root growth and fewer lateral roots than the WT under control conditions which had also been found in maize and rice (Urano et al., 2016),

whereas Bd-*clo7* mutant exhibits opposite phenotype and has approximately 12% longer primary roots and 14% longer lateral roots than wild types in control conditions. These results suggest that these two genes could act in the same pathway for the regulation of lateral root growth with *CLO7* acting as a negative regulator and Gα acts as a positive regulator. The root phenotypic analysis of the single mutants for Bd-*gα*, Bd-*clo7* and the double mutant Bd-*gα/* Bd-*clo7* in control and ABA or osmotic stress conditions in Brachypodium could give more insight on the regulation of root growth by these genes.

3.5.2. Brachypodium *CLO7* response to abiotic stress

Though the role of Brachypodium caleosins in response to abiotic stress is not well known, few studies have showed that CLO7 is transcriptionally regulated under abiotic stress conditions. Bd-*CLO7* is known to be induced more than two fold in response to cold stress for 3 hrs at 4°C and three fold in response salinity stress by 100 mM NaCl for three weeks (Hao et al., 2017; Guo et al., 2020). The microarray analysis of leaf expansion zone in response to mild stress showed that *CLO7* had minimal induction of 1.3 fold (Verelst et al., 2013). This suggests that *CLO7* in Brachypodium is involved in the abiotic stress responses. Here, we have studied the role of *CLO7* in the regulation of root growth under ABA and osmotic stress.

Lateral roots have major contribution in the total root system of the plants and hence the regulation of their development under stress conditions can contribute to the plants level of tolerance to such conditions. The lateral root traits including density and root length can regulate the drought tolerance responses in plants. Zhan et al. 2015 suggested that under water stress conditions in maize, plants with fewer and longer lateral roots can utilize the water to maximum extent from deep soil layers. Bd-*clo7* mutant had more and longer lateral root than wild type. Bd-*clo7* mutant was less sensitive to the lateral root growth inhibition in osmotic stress treatments and showed only 5% and 15% reduction in lateral root numbers and total lateral root length respectively, whereas the WT showed 42% and 54% reduction for the same traits. Experiments reported here measured the lateral root initiation and growth in seedlings up to 15 days post germination; it would be important to study the differences in lateral root growth in older field grown plants where the relationship between root number and root length may be different. These experiments also showed marked inhibition of lateral root formation by ABA, however, there were no significant differences between the WT and the Bd-*clo7* mutant in the inhibition of

lateral root growth in response to ABA treatments, which indicate that Bd-*CLO7* is not involved in the regulation of lateral root growth through ABA signalling pathway. Xiong et al. 2006 showed that the lateral root inhibition under drought stress is not fully mediated by ABA, which was demonstrated by the *abi1-1* and *aba* mutants in Arabidopsis that still showed the repression in lateral root development in response to osmotic stress. The results reported here suggest that Bd-*CLO7* in *Brachypodium* is involved in the suppression of lateral root growth under osmotic stress conditions through ABA independent signaling.

The primary root plays a vital role in embryo development and provides mechanical support to the plant in the initial growth stages for the shoot development. Another critical role of the primary root is the uptake of the water and mineral nutrients from the soil. The study in the *rtcs* mutant in maize which completely lack the lateral seminal roots and crown root showed that these plants were able to survive with only the primary roots (Hetz et al., 1996). Bd-*clo7* mutant had 12% longer primary roots than that of WT under control conditions. These longer primary roots of Bd-*clo7* mutant under control conditions can efficiently provide water and nutrients to the growing plants. In ABA treatments the primary root did not differ from that of wild type, whereas in response to osmotic stress *CLO7* slightly supressed PR, indicating that *CLO7* acts in an ABA independent regulation of this trait.

Brachypodium *CLO7* does not affect CNR induction under control condition, however in response to 150 mM mannitol, *CLO7* slightly supressed the CNRs suggesting that it does have a role in the regulation of CNRs under osmotic stress. Plants can confer drought tolerance by adaptive responses which also includes the reduction in nodal roots under water deficit conditions. This reduces metabolic cost and permits deeper axial root growth and elongation to utilize the maximum available water in deeper soil layers (Lynch et al., 2014). Bd-*clo7* mutant fails to show this response under our experimental conditions and showed growth of the CNRs under osmotic stress treatments. This suggests that under osmotic stress conditions suppression of coleoptile node roots by *CLO7* may possibly be an act of an adaptive response to drought tolerance.

3.6. Conclusion

The caleosin gene family in *B. distachyon* includes ten members. Brachypodium CLO7 has been found to interact with Bd-Gα subunit and to regulate primary root growth under control and ABA stress conditions. The Bd-*clo7* mutant analysis showed that *CLO7* supresses lateral root growth under conditions of osmotic stress in an ABA independent manner and that *CLO7* also affects coleoptile node root induction in response to osmotic stress. This study gives an insight into the role of Bd-*CLO7* in regulation of root growth in response to ABA and osmotic stress.

Supplementary Table S3.1. Full length cDNA sequences for *Brachypodium distachyon Gα* and *CLO7*

>Brachypodium-*Gα*
ATGGGCTCATCTTGCTCTAGGCCTCATCTTAATGAAGCTGAGGCTGCTGAGAACGGCAAGTCTGC
TGAGATTGATCGGAGGATCCTGCAAGAGACTAAGGCTGAGCAGCACATTCACAAGTTGCTGTTGC
TTCGATGAGGCTGAGCTTCGGTCTTACATCTCTGTGATCCACGCTAACGTGTACCAGACCATCAA
GATCCTGTACGACGGTGCTAAAGAGCTGGCTCAGGTTGAGCCTGAGTCCTCTAAGTACGTGATCA
GCCCTGACAATCAAGAGATCGGCGAGAAGATCTCTGAGATCGGTGGTAGGCTTGATTACCCTCTG
CTTTGCGAGGAACTGGTGCACGATATTAGGAAGCTGTGGGAAGATCCTGCCATCCAAGAGACTTA
CAGCAGGGGTTCTATTCTCCAGGTTCCAGATTGCGCCCAGTACTTCATGGAAAACCTTGATAGGC
TGGCCGAGGCTGATTACGTGCCAACAAAAGAGGATGTTCTGCACGCTAGAGTGAGGACTAATGGC
GTGGTGGAAATCCAGTTCTCTCCACTTGGTGAGTCAAAGAGAGGTGGCGAGATCTACAGGCTGTA
CGATGTTGGTGGTCAGAGGAATGAGCGGAGGAAGTGGATTCATCTGTTCGAGGGTGTTGACGCTG
TTGTGTTCTGCGCTGCTATCTCTGAGTACGACCAGATGCTTTTCGAGGACGAGGCTCAGAACCGG
ATGATGGAAACAAAAGAACTGTTCGACTGGGTGCTGAAGCAGCGGTGCTTTGAAAAGACCAGCTT
CATGCTGTTCCTCAACAAGTTCGACATCTTCGAGCGGAAGATCCAGAAGGTGCCACTTACTGTTT
GCGACTGGTTCAAGGACTACCAGCCTATTGCTCCTGGTAAGCAGGATGTTGAGCACGCTTACGAG
TTCGTGAAGAAGAAGTTCGAGGAACTCTACTTCCAGTCCAGCAAGCCTGATAGGGTTGACAGGGT
GTTCAAGATCTATAGGACCACCGCTCTGGACCAAAAGCTGGTGAAGAAAACCTTCAAGCTGATCG
ACGAGAGCATGCGGAGATCTAGGGAAGAGACTTGA

>Brachypodium-*CLO7*
ATGGCTAGCAAGTCTGCTAACACCGAGGGCATCAAGAAAGAAAGCGAGGCTGACGTGTACAACTC
TGAGCTTACACCTCTTCAGAGGCACGTGGCATTCTTCGATAGGAACAAGGACGGCATCATCTACC
CGTCTGAGACTTACGAGGGTTTCAGAGCTATTGGTTGCGGTGTGGCTCTGTCTGCTTTCAGCGCT
GTGTTCATTAACGGTCTGCTTGGCCCTAAGACCATTCCTGAGAATGAGAAGGCTGCCGCTTTCAA
GTTCCCGATCTACGTGAAGAACATCCACAAGGGTAAGCACGGCTCTGATAGCGGTGTGTACGATT
CTCATGGTCGTTTCGTGCCTGAGAAGTTCGAAGAGATCTTCAAGAAGCACGCTCACACCAGGCCT
GATGCTCTTACTGGTAAAGAGCTTCAAGAGCTGCTGCAGGCTAACAGAGAGCCTAACGATCTGAA
GGGTAGAGTTGGTGGTTTCACCGAGTGGAAGGTGTTGTACTCTCTGTGCAAGGACAAAGAGGGCT
ACCTGCACAAAGAGACTGTCAGGGCTGTTTACGACGGTAGCCTTTTCGTGAAGCTTGAGCAAGAG
CGGAAAGAGGCTAAAGAGTCCGCCAAGAAGAAGTAG

Chapter 4: Brachypodium *CALEOSIN 3* modulates root growth under abscisic acid and osmotic stress

4.1. Abstract

In Arabidopsis *Clo3* is involved in the ABA and osmotic stress responses. Since the roots are the first sensors of osmotic stress, here we have characterized the role of Brachypodium *CALEOSIN3* in the regulation of root growth under ABA and osmotic stress responses. It was found that Bd-*CLO3* acts as a positive regulator of primary root growth under control, ABA and osmotic stress conditions. Bd-*CLO3* positively regulates lateral root growth under control conditions, whereas it acts as a negative regulator of lateral root growth in response to ABA treatment. The significant inhibition of lateral root growth under mannitol treatments showed that Bd-*CLO3* has moderate role in inhibition of lateral root elongation under osmotic stress and it may mediate regulation through ABA dependent and ABA independent pathways. Unlike work in Arabidopsis, the G protein alpha subunit of Brachypodium, Bd-Gα did not show any *in vivo* interaction with full length CLO3 or N- terminal end truncation or C-terminal end truncations of CLO3. This work suggests that the interaction for the Gα and CLO3 in not conserved in Brachypodium. However, like other caleosins Bd-CLO3 was found to be localized to the endoplasmic reticulum.

4.2. Introduction

The alarming increasing human population and unfavourable environmental conditions will be the two major hindrances to satisfy the demand for the food in the near future. The minimization of the crop losses caused by environmental stress will surely play an important role in contributing to future food security. Among these environmental stress conditions, drought stress is one of the complex and important abiotic stresses that cause losses in the crop productivity and yield. The responses of plants to this stress function at different levels including morphological, physiological and biochemical, and in the different plant parts that include shoot as well as the below ground root system. The effect of the drought stress in cereal crops is exemplified by the study of Daryanto et al. 2016 which showed that the 40% reduction in the water supply cause yield reduction of 20% and 39% in wheat and maize respectively. Nevertheless, the plants possess mechanisms to cope with drought stress which can be divided into three categories. The

first one is drought escape where plant shortens its life cycle, second is drought avoidance that includes adjustments in the root system such as growing deeper roots, deposition of wax on leaves and closing of stomata to avoid the transpiration losses under drought stress conditions, and the third one is drought tolerance through the production of osmolytes and antioxidants (Levitt, 1980). The roots are the first plant organs that can serve as the receiver of the first signal of soil drying and activate signalling pathways involved in drought tolerance (Schachtman and Goodger, 2008). However, it is a difficult task to study the whole below ground root system of the plants due to its complex and fragile nature of the different types of roots especially in the early stages of plant development. Lateral root growth response under osmotic stress is an important aspect in drought tolerance mechanisms. In osmotic stress conditions, the adjustment in the lateral root elongation zone, often by repressing lateral root development and enhancing primary root elongation, is associated with drought tolerance and maintenance of turgor pressure under drying soil conditions to utilize the maximum of the available water (Azhiri-Sigari et al., 2000).

Deak and Malamy, 2005 studied the effect of the osmotic stress of 60 mM mannitol on the repression of total lateral root length in abscisic acid (ABA) deficient mutants *aba2-1* and *aba3-1*, in Arabidopsis and showed that in both ABA deficient mutants total LR lengths were reduced by 52.2% and 28.4%, respectively, in 12 day old seedlings, whereas in WT lateral roots were repressed by 97.7% under the same conditions. This demonstrated that ABA plays the role in the repression of lateral root elongation under osmotic stress. In barley roots, ABA levels were increased four fold under drought stress conditions and even transient water deficit induced the ABA and auxin responsive genes in roots (Orman-Ligeza et al., 2018). The treatment with 1 mM auxin rescued and promoted the root growth under osmotic stress, whereas ethylene did not affect the regulation of root growth under osmotic stress (Rowe et al., 2016). This indicates that the interplay between ABA and auxin can determine the fate of lateral root growth under osmotic stress. Xiong et al. 2006 in the study with *dig* mutants in Arabidopsis showed that the lateral root inhibition in response to the drought stress is an adaptive response because the *dig* mutants with enhanced inhibition of lateral roots under ABA treatment were drought tolerant, and also grew well under control conditions.

The increase in ABA levels under stress conditions is a known phenomenon. The MYB77, MYB44, and MYB73 transcription factors in Arabidopsis, have been shown to interact with the ABA receptor PYL8 (Pyrabactin resistance 1–like) by yeast two-hybrid assay and by the luciferase assay in Arabidopsis protoplasts, and are known to be involved in the regulation of lateral root growth. The interaction of PYL8 and MYB77 was enhanced by a combination of ABA and auxin. PYL8 and MYB77 interaction promoted the lateral root growth through auxin responsive genes when plants were in the recovery phase of growth inhibition. The Electrophoretic Mobility Shift Assay showed the enhanced binding of MYB77 to the Myb-DNA binding site, MBSI motif CRGTTA in ABA independent manner. These results suggest that lateral root growth promotion by MYB77 is regulated by PYL8 through crosstalk of ABA and auxin signalling (Zhao et al., 2014). This suggests that the lateral root growth regulation is a complex phenomenon which involves ABA and auxin signalling.

Caleosins are calcium binding proteins with a single conserved EF hand domain and are known to be involved in the stress signalling. The first reported caleosin in the rice was shown to be induced in the vegetative tissues in response to ABA, dehydration and salt stress (Frandsen, 1996). Most of the studies of caleosins have focused on *Responsive to Dehydration* (*RD20*), also referred to as *Caleosin 3* (*CLO3*), in Arabidopsis, which is known to be induced by both biotic as well as abiotic stress. *RD20* had been shown to be involved in abiotic stress responses through different hormonal signaling pathways and to regulate plant growth. *RD20 i*s a well-known stress marker gene and was shown to be expressed in above ground tissues, especially in the guard cells and cells near guard cells using RD20 promoter:GUS reporter transgenic plants. It is known to be induced over 100 fold by ABA treatment and by osmotic stress. *RD20* is known to act as a positive regulator of ABA inhibition of seed germination and drought tolerance; *rd20* mutant showed lower water use efficiency and higher transpiration rates than WT under water deficit conditions (Aubert et al., 2010; 2011). *RD20* has also been associated with gibberlic acid mediated flowering in crosstalk with the other hormones, jasmonic acid and ethylene and the *RD20* overexpressor line had shown earlier flowering phenotype than control plants under short day conditions (Blée et al., 2014). In Brachypodium, *CLO3* had been found to be induced by drought and salicylic acid treatment, whereas it was downregulated under submergence conditions (Kakei et al., 2015; Gordon et al., 2014). This indicates that caleosins in Brachypodium can also be involved in the abiotic stress responses.

RD20 is known to interact physically with the alpha subunit of the heterotrimeric G proteins. An ortholog of Arabidopsis *RD20* in bread wheat, *Triticum aestivum*, Ta-*Clo3* interacts with the heterotrimeric G protein alpha subunit GA1, formerly referred to as GA3 (Khalil et al. 2011) and phosphoinositide-specific phospholipase C1 (PI-PLC1) (Khalil et al., 2011). Microarray analysis studies in the *T. aestivum* showed that *Clo3* was highly induced by cold treatment in the shoot (Monroy et al., 2007), and expression studies using RNA-Seq showed that it is repressed by PEG mediated osmotic stress in triticale seedlings (Khalil et al., 2014). These results suggest that *RD20* plays a role in ABA and osmotic stress responses possibly through the different regulatory signaling pathways including heterotrimeric G proteins and other ABA mediated pathways, and it will be important to know if the functions of these homologous genes are conserved in monocot species. The interaction between the G protein α subunit in different species with caleosins and their response to hormones like ABA in regulation of adaptive responses make us to think whether these two genes are involved in the same or different pathway which can be further studied by phenotypic analysis of the single and double mutants for these genes.

It is difficult to study the functional genomics of wheat due to its hexaploid nature and a massive genome size of 17 gigabases, thus, to overcome these difficulties, *Brachypodium distachyon* has been extensively used as a model to study the functional genomics of the grasses. Moreover, Brachypodium shares high degree of similarity in the root development and root anatomy with wheat (Draper et al., 2001; Huo et al., 2009). The availability of a large collection of T-DNA insertional mutation lines in *B. distachyon* facilitates the study of the functions of genes. The homolog for *Triticum Clo3* and Arabidopsis *RD20* had been previously identified in *B. distachyon* (Bradi1g70390.1) by our lab. Here, we carried out the characterization of protein-protein interaction of Bd-Gα and full length and truncated versions of Bd-CLO3 by BiFC and yeast two-hybrid assay. In most of the previous studies, the role of *RD20* has been studied under ABA and osmotic stress in relation to the above ground tissues. Since root architecture is a key element in a plant's capacity to access water resources, we have characterized T-DNA insertional mutant in Brachypodium *CLO3* (Bd-*clo3*) for root growth characteristics in response to ABA and osmotic stress in this study. This study will give an insight into the role of Brachypodium *CLO3* in the regulation of root growth under ABA and osmotic stress.

4.3. Material methods

4.3.1. Plant Material and Growth conditions

The T-DNA insertion mutant line for Bd-*CLO3* gene (JJ21376 with the insertion in the intronic region of the gene), in the Bd21-3 ecotype wild type background was received from DOE Joint Genome Institute, (Walnut Creek, California). Plants were grown in 3:1:1 mixture of black soil: peat moss: vermiculite. The homozygous lines for Bd-*clo3* were identified in the T3 generation by DNA extraction and screening by PCR using the primers listed in **Table 4.1** and the homozygous lines were grown for seed increase to further to carry out the experiments. Plants were grown in greenhouse under long day conditions at 21-24°C; natural lighting was supplemented with artificial illumination to maintain long day conditions of 16 hr of light and 8 hr of darkness.

Table 4.1. List of primers used in screening of Bd-*clo3* mutant

Primers name	Primer sequence	Purpose
Bd-*CLO3*FP gene	TGAAGGTGATTGGGTTTATGC	Screening for gene
Bd-*CLO3*RP gene	GGAAGGAGGGAGTATTTAGGAGTC	
Bd-*CLO3*FP2 gene	TCATAGATAAAAGAGAAGCTCGACC	Screening for T-DNA
RP T3 T-DNA LB	AGCTGTTTCCTGTGTGAAATTG	

Seeds for WT Bd21-3 and Bd-*clo3* were cold stratified in wet paper towels at 4°C for ten days to obtain synchronised germination. Seed sterilization was carried out by removal of palea and treatment for 10 min. with 2% sodium hypochlorite (diluted from 10.3 % household bleach) and 0.001% of Triton X-100; seeds were subsequently washed 4-5 times in sterilized distilled water. Seeds were germinated for three days on 150 mm MS plates with standard media containing 0.2% MS salt, 0.05% MES hydrate and 2% sucrose, adjusted to pH of 5.7 with KOH. Germinated seeds with similar primary roots were transferred to MS plates supplemented with 0.05 µM ABA or 150 mM mannitol, or to control MS plates. The seedlings were grown in E15 Conviron growth chambers at 22°C, under long day conditions (16hr light, 8hr dark) and light intensity of 110-130 $\mu E\ m^{-2}\ s^{-1}$.

4.3.2. Root Growth measurements

The root growth measurements in response to ABA and osmotic stress treatments for WT and Bd-*clo3* mutants grown on agar media were recorded for primary root lengths after day seven;

coleoptile node roots (CNR) and lateral root lengths were determined on day twelve after transfer to media with 0.05 μM ABA or 150 mM mannitol. Images were captured using Nikon digital camera and root numbers and lengths were measured by ImageJ software version 1.43.

4.3.3. BiFC and intracellular localization of Bd-CLO3

To determine the *in vivo* protein-protein interaction for Bd-Gα (Bradi2g60350.1) and Bd-CLO3 (Bradi1g70390.1), the full length cDNA clones were synthesized by GeneArt® Strings™ DNA Fragments (Carlsbad, California, United States). The *in vivo* protein-protein interaction was determined by bimolecular fluorescence complementation (BiFC) with two different pairs of YFP tagged BiFC vectors that include pBatL-B-sYFP-N and pBatL-B-sYFP-C (Grigston et al., 2008), and PCL112-nYFP and PCL113-cYFP vectors (received from Dr. Alan Jones, University of North Carolina). Three truncated versions for Bd-CLO3 were used to investigate the *in vivo* protein-protein interaction; these included the first 74 amino acids (aa) which are the N-terminal region without proline knot, the N-terminal region with the proline knot that extended up to aa 105 and the C terminal region from aa 107 to 218.

The FL coding region with versions that did or did not include the stop codon for Bd-*Gα*, Bd-*CLO3,* and three truncated versions of Bd-*CLO3* with stop codon were amplified using primers with Gateway ends and were cloned to pDONR207 entry clones using BP reaction. The entry clones carrying Bd-*Gα* and Bd-*CLO3* coding regions without stop codon were recombined with N-terminal half YFP tag, pBatL-B-sYFP-N and C-terminal half YFP tag, pBatL-B-sYFP-C vectors respectively, using Gateway LR reactions. Similarly, the entry clones carrying Bd-Gα with stop codon was recombined with half N-terminal YFP tag vector PCL112-nYFP and FL Bd-*CLO3,* and three truncations with stop codon were recombined with half C terminal YFP tag vector PCL113-cYFP using LR reaction. For intracellular localization of Bd-CLO3, the full length entry clone was recombined with GFP plant expression vector PK7FWG2 using LR reaction. The details for the primers and vectors used in cloning for BiFC constructs are given in **Table 4.2 and 4.3**.

Table 4.2. List of primers used in cloning plasmid construct for BiFC, GFP and yeast two hybrid

Primers name	Primer sequence	Purpose
Bd-*Gα* FP	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCATGGGCTCATCTTGCTCTAG	Cloning in BiFC YFP split vector PBatL
Bd-*Gα* RP	GGGGACCACTTTGTACAAGAAAGC TGGGTCAGTCTCTTCCCTAGATCTCCG	
Bd-*Gα* FP	GGGGACAAGTTTGTACAAAAAAGCA GGCTTCATGGGCTCATCTTGCTCTAG	Cloning in BiFC YFP split vector PCL and yeast two hybrid vectors
Bd-*Gα* RPST	GGGGACCACTTTGTACAAGAAAGCTG GGTATCAAGTCTCTTCCCTAGATCTCC	
Bd-*CLO3*-GWF	GGGGACAAGTTTGTACAAAAAAGCAG GCTTCATGGCTATCAGAAGGCAGCC	Cloning FL *CLO3* in BiFC YFP split vector PBatL, GFP vector PK7FWG2
Bd-*CLO3*-GWR	GGGGACCACTTTGTACAAGAAAGCT GGGTCCATAACACCCTGGCTAGAAGCC	
Bd-*CLO3*-GWF	GGGGACAAGTTTGTACAAAAAAGCA GGCTTCATGGCTATCAGAAGGCAGCC	Cloning in FL *CLO3* BiFC YFP split vector PCL and yeast two hybrid vectors
Bd-*CLO3*-GWRST	GGGGACCACTTTGTACAAGAAAGC TGGGTCCTACATAACACCCTGGCTAGAAGC	
Bd-*CLO3*-GWF	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCATGGCTATCAGAAGGCAGCC	Cloning N terminal *CLO3* truncation in BiFC vector PCL and yeast two hybrid vectors
Bd-*CLO3*-NTR	GGGGACCACTTTGTACAAGAAAGC TGGGTCTCAAGAGAAGCCGATAGCCACG	
Bd-*CLO3*-GWF	GGGGACAAGTTTGTACAAAAAAGCA GGCTTCATGGCTATCAGAAGGCAGCC	Cloning N terminal *CLO3* truncation with proline knot in PCL and yeast two hybrid vectors
Bd*CLO3*-NTPKR	GGGGACCACTTTGTACAAGAAAGC TGGGTCTCAGATAGACAGGTGAGGAAGAGG AGC	
Bd*CLO3*-GWF	GGGGACAAGTTTGTACAAAAAAGCA GGCTTCATGGCTATCAGAAGGCAGCC	Cloning C terminal *CLO3* truncation in BiFC vector PCL and yeast two hybrid vectors
Bd*CLO3*-CTR	GGGGACAAGTTTGTACAAAAAAGCA GGCTTCATGAAGGGCATGCACGGTTCT	

Table 4.3. Vectors used in cloning plasmid constructs for BiFC and yeast two hybrid assay

Vector name	Antibiotic resistance	Purpose
pDONR 207	Kanamycin	BP cloning entry vector
PK7FWG2	Spectinomycin	LR cloning GFP vector
pBatL-B-sYFP-N, PCL112-nYFP	Spectinomycin	LR cloning BiFC half N terminal split vectors
pBatL-B-sYFP-C, PCL113-cYFP	Spectinomycin	LR cloning BiFC half C terminal split vectors
PGADT7, PGBKT7	Ampicillin	LR cloning Yeast two hybrid vectors

The electrocompetent *Agrobacterium* strain AGL1 was transformed with YFP or GFP destination vectors. The individual cultures for Bd-Gα-nYFP, Bd-CLO3-cYFP, Bd-CLO3 truncations, Bd-CLO3-GFP, p19 and endoplasmic reticulum (ER) marker were grown overnight at 30°C (Nelson et al., 2007). The p19 vector was used to supress the gene silencing during gene expression in tobacco plants in response to Brachypodium Gα and CLO3. Cultures of *Agrobacterium* carrying YFP or GFP constructs and p19 were grown in 10 ml media to an OD600 of 0.5, and plasma membrane (PM) or endoplasmic reticulum (ER) markers were diluted to an OD600 of 0.01. For BiFC, cultures carrying Bd-*Gα*-n-YFP and Bd-CLO3-c-YFP (pBatL-B-sYFP or PCL vectors) or Bd-Gα-n-YFP and one of the Bd-CLO3-c-YFP truncations (PCL vectors), p19 and PM or ER markers were mixed in equal volumes to final volume of 3 ml. The mixture was centrifuged at 4000g for 20 min, the pellet was suspended in 3 ml of Agroinfiltration solution containing, 10 mM $MgCl_2$, 150 µM acetosyringone in sterilized distilled water. These mixed cultures were incubated at room temperature for 4 hrs and were used in the infiltration of the abaxial leaf surface of 4-5 week old *N. benthamiana* plants. Plants were grown at 21-24 °C under long day conditions in greenhouse and were imaged from 24 hr onwards using Olympus Fluoview FV10i confocal laser scanning microscope in Centre for Microscopy and Cellular Imaging at Concordia University. The images for YFP or GFP were collected with 510 nm and 527 nm filters and the wavelengths of 489 nm and 480 nm were used to excite GFP and YFP by laser diode respectively. The images for the mCherry red fluorescent protein fused to respective markers were collected with a 570-620 nm filter and wavelength of 559 nm was used to excite the protein.

4.3.4. Yeast two-hybrid assay

The yeast two-hybrid assay was used to study the physical interaction between Bd-Gα with FL

Bd-CLO3 and with Bd-CLO3 truncations. The entry clones carrying FL Bd-*Gα*, FL Bd-*CLO3,* Bd-CLO3 N- terminal without proline knot, Bd-CLO3 N-terminal with proline knot and Bd-CLO3 with C-terminal regions were mobilised to Gateway yeast two-hybrid vectors PGADT7 and PGBKT7 respectively in both AD and BD fusion configurations by LR reactions (Invitrogen). The details for the primers and vectors used in cloning for yeast two-hybrid constructs are given in **Table 4.2 and 4.3**. The yeast two-hybrid assay was performed by transformation of AD fusion of Bd-Gα with BD fusion of FL Bd-CLO3 or Bd-CLO3 truncations, and BD fusion of Bd-Gα with AD fusions of Bd-CLO3 or Bd-CLO3 truncations in AH109 yeast strain. The successful yeast transformation of Bd-Gα with Bd-CLO3 was confirmed by growth obtained on SC-Leu-Trp plates and the positive interaction for the same was determined by growth obtained on SC-Leu-Trp-His plates. SC-Leu-Trp-His plates supplemented with different concentrations of 3-Amino-1,2,4-triazole (0, 1 mM, 5 mM, 10 mM) were used to determine the strength of interaction.

4.3.5. Statistical analysis

Two way ANOVA was carried out to analyze the growth characteristics of WT and the Bd-*clo3* mutant in response to ABA and mannitol. The statistical difference between the genotypes for root growth in primary roots (PR) and lateral roots (LR) were analysed using one way ANOVA followed by Duncan's Multiple Range test. Mann-Whitney rank sum test was used to determine the statistically significant differences between WT and Bd-*clo3* mutant in ABA or mannitol treatment to compare the development of coleoptile node roots.

4.4. Results

4.4.1. Bd-*CLO3* positively regulate primary root growth under ABA and osmotic stress

To determine the effect of ABA and osmotic stress on primary root growth reduction, the primary root lengths were measured seven days after the transfer of seedlings to the MS treatment plates with 0.05 µM ABA, 150 mM mannitol, or control plates. The Bd-*clo3* mutant was less sensitive to the primary root growth inhibition by ABA and mannitol than the wild type. In control conditions, the WT had longer primary roots than Bd-*clo3* mutant, 5.4 cm vs 3.6 cm. Bd-*clo3* mutant had 17% and 22% reduction in the primary root length under 0.05 µM ABA and 150 mM mannitol, whereas wild type showed 35% and 30% reduction in these treatments respectively.

Two way ANOVA showed significant genotype by treatment interaction effects indicating that the mutant responded to ABA treatment and osmotic stress differently than the WT. Thus, Bd-*CLO3* positively regulates the primary root growth under control conditions and the mutation in *CLO3* partially disrupts the normal inhibition of root elongation by ABA and osmotic stress. The primary roots of the *clo3* were shorter on the stress treated plants and on those on the control media but the degree of inhibition was less than that seen in the WT. The details for the primary growth reduction under ABA and mannitol and two way ANOVA results are given in **Figure 4.1 and Table 4.4.**

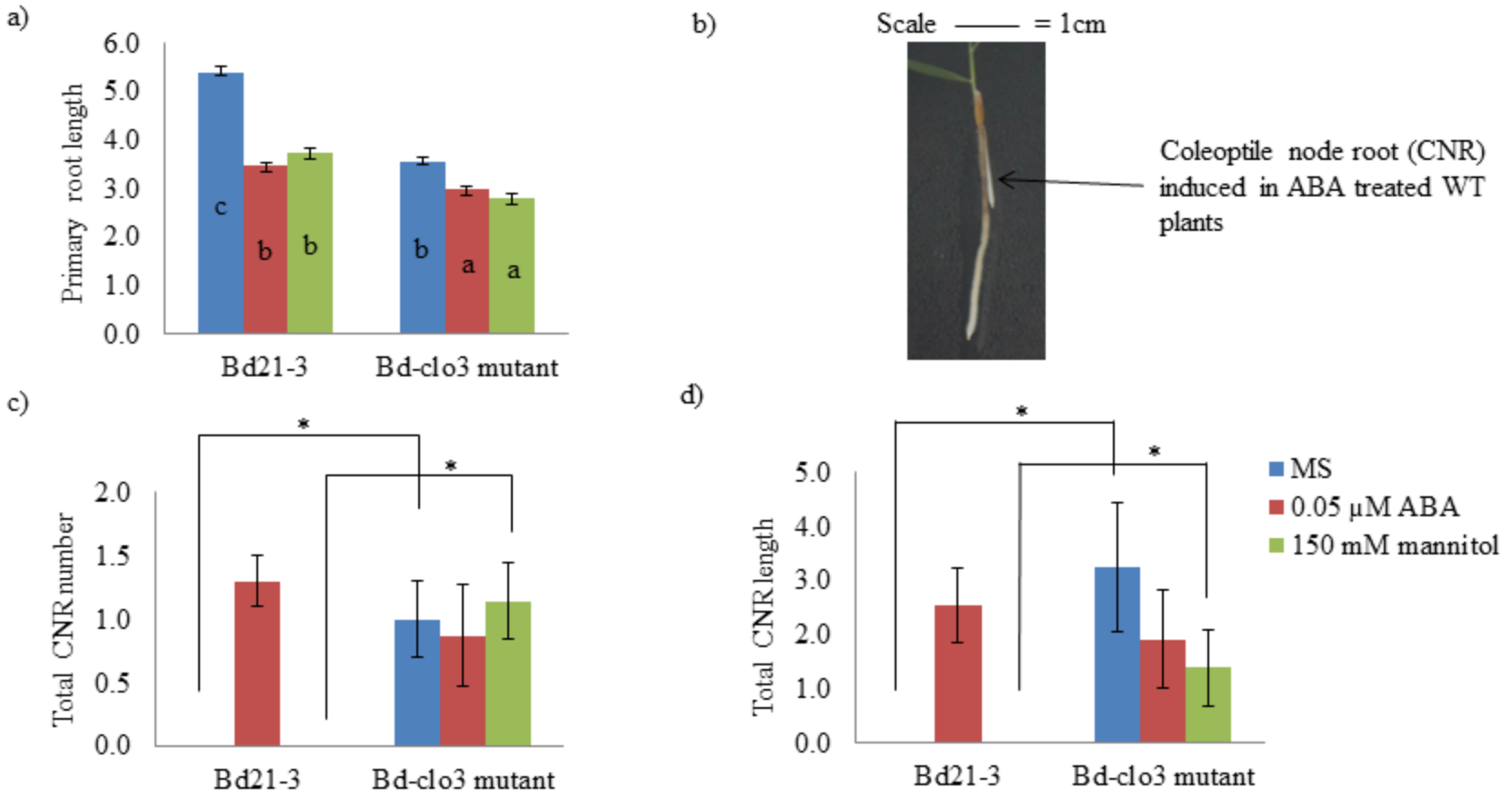

Figure 4.1. The effect of ABA and mannitol on root growth of WT Bd21-3 and Bd-*clo3* mutant. a) Primary root length of plants grown with 0.05 µM ABA or 150 mM mannitol were measured on day seven of the treatments. b) The coleoptile node roots (CNR) induced by 0.05 µM ABA treatments in WT and Bd-*clo3* mutant. The significant differences for PR are analysed by one way ANOVA ($p < 0.05$). The letters on bars indicates the ranks assigned by Duncans multiple range test, bars that share a same letter are not significantly different. The effect of 0.05 µM ABA and 150 mM mannitol on c) Total coleoptile node root (CNR) number and d) Total coleoptile node root (CNR) length measured on day 12 after ABA or mannitol treatments. Mann-Whitney rank sum test is used to determine the significant differences for CNR numbers and lengths. '*' represent treatments that are significantly different. Error bars represent standard errors of the means.

Table 4.4. Two way ANOVA for effect of 0.05 µM ABA or 150 mM mannitol on primary root growth

WT-Bd-*clo3*	p-value	
	Primary root length	
	0.05 µM ABA	150 mM Mannitol
Genotype	0.000	0.000
Treatment	0.000	0.000
Genotype × Treatment	0.000	0.000

Note: The effect of 0.05 µM ABA or 150 mM mannitol on primary root growth of WT and Bd-*clo3* mutant is determined on day 9 after the treatment. The values represent the p-values.

4.4.2. Bd-*CLO3* positively regulates coleoptile node root growth under control and osmotic stress

On day 12 of the growth under control conditions, the WT did not grow CNRs, whereas Bd-*clo3* mutant averaged 1 CNR with an average length of 3.2 ± 1.2 cm. The 0.05 µM ABA treatment induced the coleoptile node roots in WT (WT ABA treated CNRs: 1.3 ± 0.2; CNR lengths: 2.5 ± 0.7) (**Figure 4.1**), whereas ABA treatments supressed the number of CNRs and total CNR lengths by 12% and 41%, respectively, in the Bd-*clo3* mutant. Though the CNR were reduced in response to 0.05 µM ABA the in both WT and mutant, these two genotypes did not show significant differences for CNR induction under ABA treatment. The details for the effect of ABA on total CNR number and lengths are given in **Figure 4.1** and Mann-Whitney rank sum test for it is given in **Table 4.5**.

In response to 150 mM mannitol treatment, WT did not grow CNR, whereas Bd-*clo3* mutant showed 9% increase in the number of CNR and 56% reduction in total CNR lengths. The wild type and Bd-*clo3* mutant showed significant differences for CNR induction under normal and osmotic stress conditions. These results suggest that *CLO3* supresses the CNRs in response to osmotic stress conditions. The details for the effect of 150 mM mannitol on total CNR numbers and lengths is given in **Figure 4.1** and Mann-Whitney rank sum test for the effect of mannitol on CNR induction is given in **Table 4.5**.

Table 4.5. Mann-Whitney rank sum test for effect of 0.05 µM ABA and 150 mM mannitiol on total coleoptile node root growth

WT Bd21-3 compared to Bd-*clo3*	p-value	
	Total coleoptile root number	Total coleoptile root length
Control treatment	0.027	0.027
ABA treatment	0.321	0.321
Mannitol treatment	0.014	0.014

Note: The effect of 0.05 µM ABA or 150 mM mannitol on total coleoptile root numbers and lengths of WT Bd21-3 and the Bd-*clo3* mutant measured after 12 days of treatment. Values represent the p-values.

4.4.3. Bd-*CLO3* inhibits LR growth under ABA stress

The effect of 0.05 µM ABA on the reduction of lateral root growth was determined on day twelve of the treatment. In control conditions, Bd-*clo3* mutant had 36% fewer total lateral roots number and 54% less total lateral root length than the WT. The Bd-*clo3* mutant was less sensitive to lateral root inhibition by ABA than the WT; the total lateral root length reduction of 11% was found in Bd-*clo3* mutant, whereas 78% reduction in total lateral root length for WT was observed. Similarly, the WT type had 64% reduction in total lateral root numbers, whereas the mutant was insensitive to lateral root inhibition and showed no change in lateral root numbers. The genotype by treatment interaction effect in the two way ANOVA indicated that the response to ABA treatment was significantly different for total LR numbers and total LR length. These results suggest that Brachypodium *CLO3* is a positive regulator of lateral root growth in control conditions and is involved in the suppression of lateral root development through ABA mediated pathways. The details for the effect of 0.05 µM ABA on reduction of total lateral root number and lengths are given in **Figure 4.2** and two way ANOVA for it is given in **Table 4.6.**

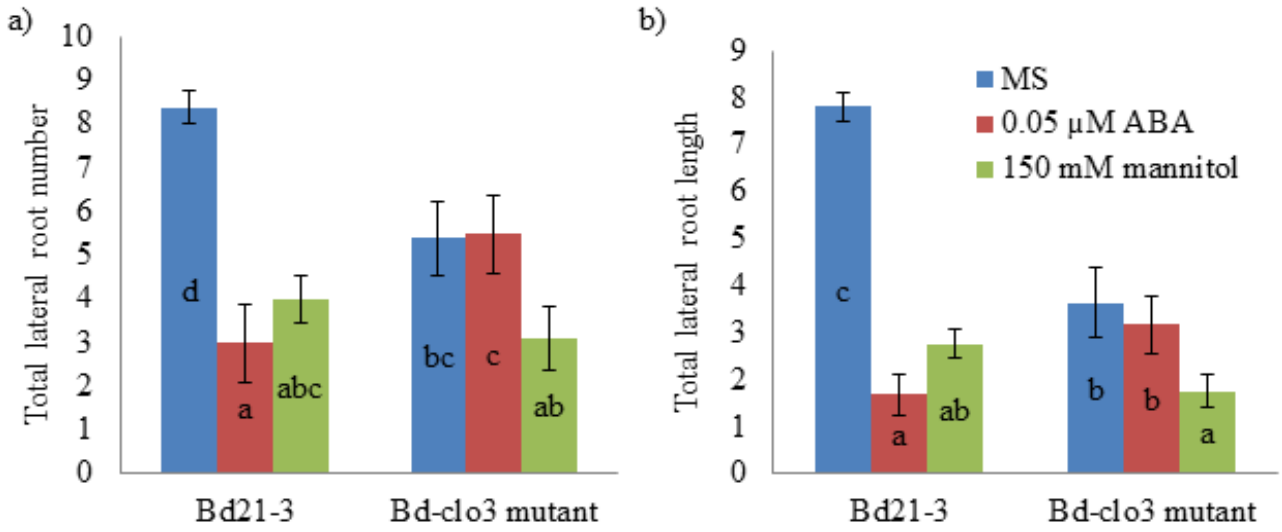

Figure 4.2. The effect of ABA and Mannitol on lateral root growth of WT Bd21-3 and Bd-*clo3* mutant. a) Total lateral root numbers and b) Total lateral root lengths of seedlings grown with 0.05 µM ABA or 150 mM mannitol were measured on day 12 of the treatments. The significant differences are analysed by one way ANOVA ($p < 0.05$). The letter on each bar indicates the ranks assigned by Duncans multiple range test and error bars represent standard errors of the means.

Table 4.6. Two way ANOVA for effect of 0.05 µM ABA on total lateral root growth

Bd21-3 compared to Bd-*clo3*	p-value	
	Total lateral root number	Total lateral root length
Genotype	0.726	0.011
Treatment	0.002	0.000
Genotype × Treatment	0.002	0.000

The effect of 150 mM mannitol on lateral root growth reduction was also observed on day twelve of the treatment. Bd-*clo3* mutant was less sensitive to the inhibition of the lateral root elongation than WT; the mutant showed 43% reduction in total lateral root numbers and 50% reduction in total lateral root lengths under osmotic stress conditions whereas WT showed the reduction of 52% and 64% for total lateral root numbers and lengths respectively. Two-way ANOVA indicated that the *clo3* mutant responded significantly differently to mannitol treatment that the WT for total lateral root length but not for the number of lateral roots. These results suggest that Bd-*CLO3* is involved in the suppression of lateral root elongation under osmotic stress. The

details for the effect of 150 mM mannitol on the reduction of total lateral root numbers and lengths is given in **Figure 4.2** and two way ANOVA for it is given in **Table 4.7.**

Table 4.7. Two way ANOVA for effect of 150 mM mannitol on total lateral root growth

Bd21-3 compared to Bd-*clo3*	p-value	
	Total lateral root number	Total lateral root length
Genotype	0.004	0.000
Treatment	0.000	0.000
Genotype × Treatment	0.086	0.001

Note: The effect of 150 mM mannitol on total lateral root numbers and lengths of WT Bd21-3 and Bd-*clo3* mutant was measured after 12 days of treatment. Values represent the p-values.

4.4.4. Protein-protein interaction and intracellular localization

Brachypodium Bd-Gα did not show interaction with full length Bd-CLO3 or Bd-CLO3 truncations when assayed by BiFC, nor when the protein-protein interaction was assayed by yeast two-hybrid assay. These results suggest that Brachypodium Gα does not interact either with Bd-CLO3 nor its N or C-terminal regions *in vivo*. The full length GFP tagged Bd-CLO3 expression analysed in the 4-5 week old *N. benthamiana* assayed 42 hrs after agroinfiltration showed that Bd-CLO3 was localized to the endoplasmic reticulum (ER) as seen by network like structure of ER and overlap of GFP with mCherry endoplasmic reticulum marker. The detail for the intracellular localization of Bd-CLO3 is given in **Figure 4.3**.

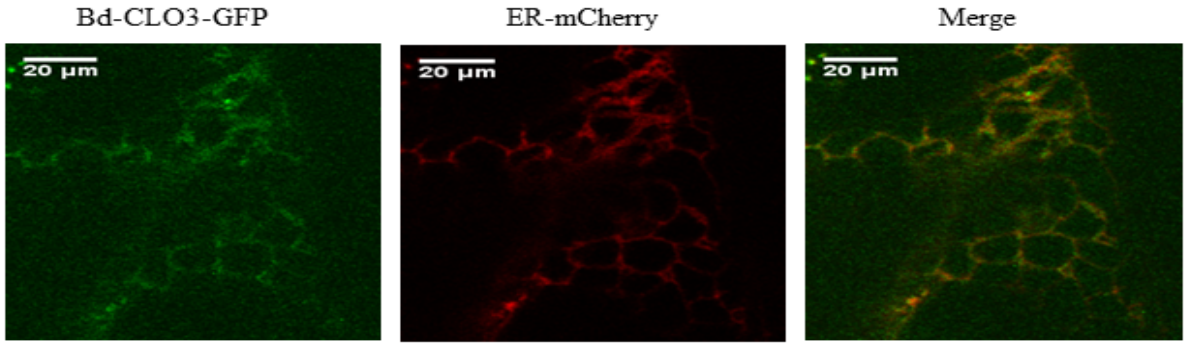

Figure 4.3. Intracellular localization Bd-CLO3 to endoplasmic reticulum. The Bd-CLO3-GFP expression was analysed in the 4-5 week old *N. benthamiana* epidermal leaf tissues 42hrs after Agrobacterium infiltration. ER-mcherry is the endoplasmic reticulum marker; the merged image shows the overlap between Bd-CLO3-GFP and ER marker; Scale bar = 20 µM.

4.5. Discussion

4.5.1. Bd-CLO3 in regulation of root growth under ABA and osmotic stress

Among the caleosin gene family members studied in Arabidopsis At-*Clo3* and At-*Clo4* had been shown to be involved in ABA and osmotic stress signalling pathways. In response to stress conditions, the increased cytosolic Ca^{2+} has been known to bind the different calcium binding proteins. Caleosins are also one the calcium binding proteins known to be involved in stress responses. There is possibility that the increased Ca^{2+} levels also recruits the caleosins to regulate the stress responses. The ABA mediated responses for caleosins that were studied through the characterization of mutants included seed germination, stomatal regulation and drought tolerance responses (Aubert et al., 2010; Kim et al., 2011). *RD20* is known to be expressed throughout the plant development and under non stress conditions and to have increased expression in response to stress in the above ground aerial tissues and ongoing investigations in the Gulick lab have characterized *RD20* induction in roots in response to stress and ABA treatment. The microarray analysis of severely drought stressed Brachypodium plants showed that *CLO3* was induced by more than five fold in the leaf tissue analyzed (Verelst et al., 2013). This led us to investigate whether Bd-*CLO3* is involved in the root growth regulation under ABA and drought stress. The Bd-*clo3* mutant had shorter root system, shorter primary roots and lateral roots compared to the WT in control conditions; however it showed more and longer CNRs. This indicated that *CLO3* is a positive regulator of lateral root growth under control conditions. The lower sensitivity of the Bd-*clo3* mutant in the lateral root growth inhibition in response to 0.05 μM ABA suggests that Bd-*CLO3* is involved in the ABA mediated inhibition of lateral root growth. In response to 150 mM mannitol treatment, Bd-*CLO3* was more sensitive to the lateral root inhibition than in ABA treatments, indicating that in addition to ABA mediated lateral root inhibition CLO3 also acts in ABA independent pathways. Taken together these results suggest that Bd-*CLO3* acts as a positive regulator of primary root length and lateral root development and is involved in the ABA and osmotic stress mediated inhibition of these roots.

In control conditions, Bd-*clo3* mutant developed CNR whereas the WT did not. The mutant also developed CNR with ABA and mannitol treatments similar to those of the plants grown on control media, whereas the WT developed CNR with ABA treatment but not with mannitol. Our results suggested that Bd-*CLO3* negatively regulates the CNR under control condition and plays

a role in induction of CNR in response to ABA. The difference between the *clo3* mutant and WT in their mannitol responses is different from their response to ABA, suggesting that the regulation of CNR under osmotic stress by *CLO3* is not mediated through ABA.

The lateral roots of wild type plants were more sensitive to the inhibition by ABA or osmotic stress treatments and repressed LR elongation by 78% or 64% respectively, whereas the primary roots were supressed by 35% and 30% under ABA and osmotic stress. These results were consistent with the study by Duan et al. 2013, which showed that lateral roots are more sensitive to the inhibition under ABA and osmotic stress than primary roots. The degree of inhibition for primary root and lateral roots found in Bd-*clo3* mutant under ABA or osmotic stress was less than wild type. The mutant was insensitive to the lateral root inhibition in response to ABA treatment and the suppression of lateral root elongation was found to be minimal for the same, which indicates that CLO3 plays a role in the ABA mediated inhibition of lateral root development. *CLO3* suppressed CNR formation under both control and osmotic stress treatments, whereas under ABA, it stimulates the CNR formation to a small degree. This indicates the complexity in the regulation of CNR growth compared to the primary and lateral root growth under ABA and osmotic stress. Bd-*CLO3* is a negative regulator of CNR growth under control and osmotic stress conditions but not under ABA stress.

4.5.2. Bd-CLO3 interaction with Bd-Gα

Though our lab has found the interaction of heterotrimeric G protein alpha subunit in *Triticum aestivum* GA3 and Ta-CLO3, and Arabidopsis GPA1 and At-Clo3, we did not find the interaction of Brachypodium Gα and CLO3. Neither the full length CLO3 nor its N- or C-terminal truncations have been found to interact with the by BiFC or yeast two-hybrid. The sequences for Brachypodium and wheat share the similarity in the coding regions. This suggests that in the protein-protein interaction of CLO3 and the alpha subunit of the heterotrimeric G protein in Brachypodium behave differently from other plant species and they do not interact with each other. The localization of Bd-CLO3 localization to the endoplasmic reticulum did agree with the localization of caleosins *Clo3* and *Clo4* from Arabidopsis.

The mutant for Bd-*gα* is not available in Brachypodium. The lateral root phenotype of Bd-*clo3* mutant and the Arabidopsis G protein alpha subunit mutant *gpa1* are similar which shows the

reduced lateral roots in both of these mutants. However, in response to ABA treatment LR inhibition in these mutants exhibit opposite phenotype, Bd-*clo3* mutant is less sensitive to the ABA mediated inhibition of LR whereas *gpa1* has stronger inhibition of LR compared to the wild type which suggests that under ABA stress these two genes behave differently. However, the study with the single Bd-*gα* or Bd-*clo3* mutant or Bd-*gα*/Bd-*clo3* double mutant will clarify the role of the genes weather they act in same or parallel pathway in the regulation of root growth.

4.6. Conclusion

Brachypodium CLO3 behaves differently than those of Arabidopsis or *T. aestivum* and does not appear to physically interact with Bd-Gα. The Bd-*clo3* mutant has shorter root phenotypes for primary and lateral root under control conditions; however it has longer CNRs under the same conditions. Bd-*CLO3* supresses CNR under control and osmotic stress conditions and is involved in the suppression of lateral root growth through ABA dependent pathways and the inhibition of lateral root elongation by *CLO3* possibly operates through both ABA dependent and ABA independent signalling.

Chapter 5: Conclusion

The dramatic contrast between the multiplicity of genes encoding heterotrimeric G proteins in animals and plant genomes raises the question of how diversity of signaling and responses to different environmental and physiologic conditions have been developed in plants. Genes encoding Gα and Gβ subunits comprise multigene families in animals with the human genome serving as a typical example with genes encoding 23 Gα, 6 Gβ and 12 Gγ subunits. However, dicots like Arabidopsis and monocots like Triticum and Brachypodium possess single Gα and Gβ subunits per haploid genome. In plants, the numbers of genes encoding Gγ subunits have been reported to range from three to ten (Trusov et al., 2012; Choudhury et al., 2011). Gγ are more diverse and are classified into three different types based on their C-terminal end composition. Though most studies showed that Gα is involved in the traits that regulate the plant developmental processes and stress responses, the multiplicity of Gγ's and their varied expression pattern in tissues and in response to abiotic stress suggests a potential diversity of roles in the regulation of traits under normal growth conditions as well as in stress responses. The large differences in the size and the sequence of the N- and C-terminal portions of the Gγ proteins indicate a divergence of function, a notion that is supported by divergence of the patterns of expression. The wheat Gγ1 was highly expressed in root, whereas Gγ3 and Gγ4 were highly expressed in stem and inflorescence tissues. Gγ1 response to stress conditions including osmotic, heat, combined osmotic and heat, and in response to infection by *Fusarium graminearum* suggest its involvement in more than one kind of stress conditions. Downregulation of Gγ3 under heat stress and Gγ4 under combined osmotic and heat stress coupled with their large differences in protein sequences suggest that they may play different roles the stress response. Gγ2 is known to be involved in hormonal signalling pathway that include auxin and ABA (Subramaniam et al., 2016). The highly expressed Gγ1 has 98 amino acids, whereas the Gγ2, Gγ3 and Gγ4 are 141, 169 and 305 amino acids respectively. The N- and C-terminal extensions of these paralogs generate a potential for a wider array of interaction with other proteins. The diversity of Gγ's in plants species possibly evolved to facilitate signalling pathways that include hormonal and stress signalling where different Gγ's can also be involved. The relation between different Gγ's and their response to given stress conditions is a promising area of study. The possible roles in abiotic and biotic stress responses of heterotrimeric G protein in monocot species need the further investigations by characterizing mutants.

Caleosins are associated with lipid droplets and have role in the structural maintenance of lipid bodies in plants. These proteins are also known as peroxygenases because they carry out the lipid transformations, which involve the oxygenation of unsaturated fatty acids to epoxy fatty acids that produces the compounds which are involved in plants stress responses. Caleosin gene family members in Arabidopsis and *Triticum aestivum* were previously shown to interact with the Gα subunit and are localized to the plasma membrane and endoplasmic reticulum (Wang, 2009; Khalil et al., 2011). Brachypodium has ten caleosin genes and this study has found that CLO3 and CLO7 are localized to the endoplasmic reticulum and the interaction of CLO7 with Gα is localized to the plasma membrane, whereas Bd-CLO3 does not interact with the Bd-Gα. This study concludes that Bd-Gα and Bd-CLO7 interaction is conserved among monocot and dicot species. The lack of interaction of Bd-CLO3 with Bd-Gα is surprising since this interaction was observed previously in both dicot and monocot species, which indicates a strong evolutionally conservation. *CLO7* has dominant role in the regulation of lateral root growth and it acts as negative regulator of LR development under osmotic stress through ABA independent signalling, whereas *CLO3* has dominant role in ABA dependent suppression of LR growth. This indicates that the caleosin gene family members CLO3 and CLO7 in Brachypodium are involved in the regulation of root growth through different pathways which include hormonal and drought stress signalling. Lateral root development has been shown to be influenced by the members of the heterotrimeric G protein complex in Arabidopsis (Urano et al., 2016) and the possibility of the regulation of root growth by Bd-Gα and Bd-CLO7 under ABA or osmotic stress can be investigated further by comparing the response of Bd-*gα* and Bd-*clo7* double mutants to single mutants. Though a mutant in Bd-Gα was not available in the mutant set reported by Bragg et al. 2012, CRISPR technology offers an avenue to develop such lines. The regulation of coleoptile nodal root growth under ABA and osmotic stress is different and rather complex. Brachypodium CLO3 and CLO7 have redundancy in the suppression of CNR under osmotic stress. Altogether this study concludes that caleosin genes family members CLO3 and CLO7 are involved in regulation of root growth through ABA dependent and ABA independent signalling.

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