Charaterization of the Heterotrimeric G-Protein Complex and Its Regulator from the Green Alga Chara braunii Expands the Evolutionary Breadth of Plant G-Protein Signaling1[C][W][OPEN]

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The lack of heterotrimeric G-protein homologs in the sequenced genomes of green algae has led to the hypothesis that, in plants, this signaling mechanism coevolved with the embryophytic life cycle and the acquisition of terrestrial habitat. Given the large evolutionary gap that exists between the chlorophyte green algae and most basal land plants, the bryophytes, we evaluated the presence of this signaling complex in a charophyte green alga, Chara braunii, proposed to be the closest living relative of land plants. The C. braunii genome encodes for the entire G-protein complex, the Gα, Gβ, and Gγ subunits, and the REGULATOR OF G-PROTEIN SIGNALING (RGS) protein. The biochemical properties of these proteins and their cross-species functionality show that they are functional homologs of canonical G-proteins. The subunit-specific interactions between CbGα and CbGβ, CbGβ and CbGγ, and CbGα and CbRGS are also conserved, establishing the existence of functional G-protein complex-based signaling mechanisms in green algae.

Heterotrimeric G-proteins comprising of Gα, Gβ, and Gγ (G-proteins hereafter) are key signaling intermediates in all eukaryotes. The catalytically active protein of the complex, Gα, can bind GTP or GDP. GDP-bound Gα remains associated with Gβγ (GDP-Gαβγ) and represents the inactive signaling status. A signal-dependent exchange of GTP for GDP on Gα causes the formation of Gα-GTP and releases the Gβγ dimer. Both these entities can interact with a variety of effectors to transduce the signal. The intrinsic GTPase activity of Gα causes hydrolysis of the bound GTP and regenerates GDP-Gαβγ, completing one signaling cycle (Cabrera-Vera et al., 2003; Offermanns, 2003; Urano et al., 2013). REGULATOR OF G-PROTEIN SIGNALING (RGS) domain-containing proteins, which enhance the GTPase activity of Gα, accelerate the rate of the G-protein cycle (Siderovski and Willard, 2005).

The core G-protein components and their biochemical properties are largely conserved across the eukaryotic clade; however, important quantitative and qualitative differences emerge when comparing the well-characterized metazoan G-protein systems with those of plants (Anantharaman et al., 2011; Bradford et al., 2013; Urano et al., 2013). For example, there is a lineage-specific expansion of individual G-protein components in metazoans, where each protein is represented by multiple members (e.g. 23 Gα, five Gβ, 12 Gγ, and 37 RGS proteins in humans) that have distinct biochemical properties (Anantharaman et al., 2011). In plants, the analysis of G-protein functions in Arabidopsis (Arabidopsis thaliana), rice (Oryza sativa), and soybean (Glycine max) has emphasized the critical roles they play in plant growth, development, and stress responses (Li et al., 2012; Zhang et al., 2012; Choudhury and Pandey, 2013; Urano et al., 2013); however, the genomes of most plants encode for a single canonical Gα and Gβ protein and few Gγ proteins (Urano et al., 2013), with the exception of plants where recent whole-genome duplications have resulted in their higher numbers (e.g. four Gα, four Gβ, and 10 Gγ proteins in soybean; Bisht et al., 2011; Choudhury et al., 2011; Pandey, 2011). Furthermore, at the biochemical level, the higher plant Gα proteins have significantly slower GTPase activity compared with the mammalian Gα proteins (Jones et al., 2011; Choudhury et al., 2012; Urano et al., 2012).

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Identification of the evolutionary origin of such critical differences is an active area of research. The available data point to the existence of rather complex and divergent regulatory mechanisms in plants compared with the established paradigms (Bradford et al., 2013; Urano et al., 2013). Specifically, many of the integral components of the G-protein complex seem to be missing from the basal plant lineages. The liverwort *Marchantia polymorpha* contains the sequence homologs of Ga, Gβ, and Gγ proteins but not the regulatory protein, RGS. Interestingly, the Ga protein from *M. polymorpha* displays rapid GTP binding (similar to plants) but also rapid GDP release (similar to metazoans; Urano et al., 2012). Remarkably, the moss *Physcomitrella patens* encodes for the Gβ and Gγ proteins but no canonical Ga or RGS proteins. The fully sequenced genomes of chlorophyte green algae, *Volvox carteri*, *Chlamydomonas reinhardtii*, *Coccomyxa subellipsosoida C-169*, *Micromonas pusilla* CCMP1545, *M. pusilla* RCC299, and *Ostreococcus lucimarinus*, do not contain G-proteins homologs (our analysis; www.phytozome.net). These observations have led to hypotheses that G-protein signaling in the green plant lineage evolved with the evolution of land plants or, alternatively, with the evolution of a sporophytic lifestyle (Urano et al., 2012, 2013; Bradford et al., 2013). It has also been reasoned that G-protein signaling in plants is independent of a receptor-dependent regulation because sequence homologs of G-protein-coupled-receptor-like proteins from flowering plants are present in green algae, even though they do not encode for G-protein components themselves (Bradford et al., 2013; Urano et al., 2013).

To address the evolutionary origin of heterotrimeric G-protein signaling in plants and its relationship to the evolution of land plants and/or the sporophytic lifestyle, we have analyzed the transcriptome of *Chara braunii* (order, Charales; class, Charophyceae). We identified a complete repertoire of a G-protein heterotrimer as well as a homolog of an N-terminal transmembrane-containing RGS protein in *C. braunii*. The proteins exhibit biochemical properties similar to their homologs in Arabidopsis. These findings challenge the established notions regarding the evolution of G-protein signaling in plants and confirm that the origin of this conserved signaling mechanism in plants is more ancient than was previously proposed.

RESULTS AND DISCUSSION

The Genome of *C. braunii* Encodes for a Complete Repertoire of Heterotrimeric G-Protein Components

A large evolutionary gap exists between the chlorophyte green algae, which do not seem to possess the G-protein components, and the most ancient land plants, the bryophytes, which contain the Gβ and Gγ proteins. The chlorophyte green algae are especially interesting from an evolutionary perspective, since they are the closest living relatives of the ancestors of the embryophytes (Becker and Marin, 2009; Niklas and Kutschera, 2010; Wodniok et al., 2011; Laurin-Lemay et al., 2012; Timme et al., 2012; Raven, 2013; Zhong et al., 2013). Superficially, these algae (e.g. the genera *Chara* and *Nitella*) have morphologies similar to basal land plants, with the presence of rhizoids, a main axis that is differentiated into nodes and giant cylindrical internodes, dimorphic branches, and whorls of leaf-like giant cells. However, in contrast to embryophytes, the plant is a gametophyte with the diploid generation represented only by the zygote, similar to other algae.

To evaluate the extent to which the existence of plant G-proteins is correlated with the evolution of embryophytes, a search was performed in *C. braunii*, a representative of the order Charales. RNA-Seq data generated from the *C. braunii* thalli at the vegetative growth phase were used for BLAST search with the Arabidopsis Ga (AtGPA1), Gβ (AtAGB1), Gγ (AtAGG1, AtAGG2, AtAGG3), and AtRGS1 genes. This analysis identified a potential homolog for each of the genes, with the exception of AtAGG3, along with some hits with high similarity to *Dictyostelium* species transcripts. To verify the accuracy of the assembled nucleotide sequences and their origin from the *C. braunii* genome, the corresponding transcripts were PCR amplified from complementary DNA (cDNA) pools generated from independent *C. braunii* cultures. Full-length cDNAs were sequenced and analyzed for the characteristic features of G-protein subunits. *C. braunii* G-protein genes showed an overall nucleotide sequence similarity of 53.1%, 48.1%, and 42.2%/39.2% with Arabidopsis GPA1, AGB1, and AGG1/AGG2, respectively. No sequence homologs were identified for AtAGG3 genes in *Chara* species, which is not surprising, as sequence homologs of AtAGG3 are present only in higher plants (Chakravorty et al., 2011; Li et al., 2012; Trusov et al., 2012). Conceptually translated amino acid sequences of *C. braunii* Ga, Gβ, and Gγ homologs (named CbGa, CbGβ, and CbGγ, hereafter) exhibit 47.9%, 43.9%, and 18.8%/23.1% identity (66.1%, 58.4%, and 28.6%/41% similarity) with the corresponding Arabidopsis proteins.

Even though we have identified only a single homolog of each of the G-protein subunits and the RGS protein, the possibility that additional homologs of these genes exist in the *C. braunii* genome cannot be ruled out at this stage. It should be noted, however, that most plant species, including moss species, the genera *Selaginella*, *Adiantum*, and *Pinus*, Arabidopsis, and rice, possess only a few, if not only one, homologs of G-protein genes, and multiplicity is typically present with respect to the Gγ proteins (Urano et al., 2012). Moreover, one type of Gγ protein seems to be specific to higher plants. The only plants that have multiple homologs of each of the subunits are the ones that have undergone recent whole-genome duplication events, such as soybean, which has four Ga, four Gβ, and 10 Gγ proteins (Bisht et al., 2011; Choudhury et al., 2011). A complete genome sequence assembly will be required to assess the existence of additional G-protein
genes in the *C. braunii* genome. Nonetheless, these data clearly show that sequences encoding for each of the functional subunits of a complete heterotrimeric G-protein complex exist in green algae.

The signature motifs required for GT binding and GTase activity, designated as G1, G2, G3, G4, and G5 domains in Ga proteins, are highly conserved in CbGa, with few similar amino acid substitutions, compared with AtGPA1 (Fig. 1A). Additional critical motifs present in Ga proteins, such as an ADP ribosylation site and an invariant Gln (corresponding to position 222 in AtGPA1) that affects its GTase activity (Oki et al., 2005), are also conserved in CbGa. Homology modeling of the three-dimensional structure of CbGa, based on the published crystal structure of AtGPA1 (Jones et al., 2011), shows that the G1 to G5 domains of CbGa attain a spatial orientation similar to that of AtGPA1 and predict the formation of a functional GT-binding pocket.

The N-terminal region of CbGa shows some variation from other known plant Ga proteins. All known higher plant Ga proteins contain a conserved Met that is a part of the signature sequences for palmitoylation (MGXXXS) and myristoylation (MGXXCS) modifications. These lipid modifications are required for the proper targeting of Ga proteins to the plasma membrane. This conserved Met also represents the translational start codon for a number of Ga proteins (e.g. in Arabidopsis). However, an additional eight (e.g. in soybean) or 10 (in most monocots) amino acids preceding this Met are also common (Bisht et al., 2011). The significance of such modifications and whether they result in alternatively translated Ga proteins in planta remain unknown. Interestingly, CbGa contains a 26-amino acid overhang from the conserved Met (Fig. 1A). However, the sequence motifs for palmitoylation and myristoylation are not present at the CbGa N-terminal region. A similar lack of these lipid modification motifs is also obvious in the Ga protein sequence from *M. polymorpha*, although it does not have any N-terminal overhangs (Fig. 1A). Whether these alterations affect the localization of basal Ga proteins or have any effect on their biochemical or signaling activity remains unknown at this time. The possibility that CbGa and MpGa remain plasma membrane localized by additional modifications (preylation of Gγ proteins) or protein-protein interactions (interaction with the RGS protein or with the βγ dimer) also cannot be ruled out.

CbGa shows ~45–90% sequence homology with Ga proteins from other plants (Supplemental Table S1). Phylogenetic analysis using Ga sequences from species representing important taxa during plant evolution resulted in clear clusters of monocotyledons and eudicotyledons that cluster with gymnosperms, which is consistent with the evolution in land plants. Outside the seed plants, the liverwort *M. polymorpha*, the spike moss *Selaginella moellendorffii*, and *C. braunii* Ga were nested without strong support (Fig. 1B).

CbGβ shows moderate (approximately 44%) sequence identity with Gβ proteins of other plant species, including the two *P. patens* Gβ proteins. The protein has seven WD-repeat motifs, typical of all Gβ proteins. Sequences important for interaction with Ga and Gγ proteins are mostly conserved in CbGβ and AtAGB1 (Supplemental Fig. S1).

The sequence identity between AtAGG1/AtAGG2 and CbGγ is relatively modest (approximately 19% and 23% with AGG1 and AGG2, respectively), which is typical of most Gγ proteins, given the large variability observed between them (Choudhury et al., 2011). However, signature motifs predicted to be essential for Gγ function, namely the coiled-coil domain at the N terminus, the DPLL motif, and the sequences predicted to be involved in its high-affinity interaction with the Gβ proteins, are mostly conserved in CbGγ. The protein shows relatively higher homology (27.9% identity) with the *P. patens* Gγ1 protein (Supplemental Fig. S2).

None of the basal plant lineages, including *P. patens* and *M. polymorpha*, encode for an RGS protein homolog. Furthermore, many monocots do not possess an RGS protein homolog, and it is predicted to have been lost multiple times during evolution (Urano et al., 2012). Remarkably, a sequence homolog of *AtRGS1* is present in the *C. braunii* transcriptome. The protein (1,097 amino acids) is encoded by a 3.3-kb transcript and is almost twice the size of other known plant RGS proteins (Choudhury et al., 2012). The N-terminal part of the protein is predicted to possess nine membrane-spanning helical domains, in comparison with seven in Arabidopsis and soybean, and an RGS domain at the C-terminal region (Fig. 1C). Comparison of the nucleotide sequences of the full-length CbRGS and AtRGS1 genes showed only 22.9% identity, while the corresponding amino acid sequences revealed 12.9%/21.8% identity/similarity (Supplemental Fig. S3). Relatively higher identity/similarity (approximately 30%/50%) was observed between the RGS domains of CbRGS and AtRGS1. Interestingly, CbGa contains the conserved Thr residue (Thr-194 of AtGPA1), proposed to serve Thr residue (Thr-194 of AtGPA1), proposed to be critical for its regulation by RGS protein activity (Urano et al., 2012). Phylogenetic analysis of CbRGS with different plant RGS proteins (Fig. 1D) revealed clear monophyly of monocots, flowering plants, and seed plants. The branch length leading to *C. braunii* being slightly deeper than *S. moellendorffii* is consistent with orthologous relationships of these genes.

### *C. braunii* G-Protein Components Are Transcriptionally Active

To confirm the transcriptional activity of the sequence homologs of G-protein genes, additional expression analyses by real-time quantitative PCR were performed, using cDNA generated from the internodes and dimorphic branches of *C. braunii*. All G-protein genes and CbRGS exhibited a low, but unambiguously detectable, expression, with no significant differences in expression levels between the internodes and dimorphic branches (Fig. 2). This is similar to the
Figure 1. Ga and RGS proteins from C. braunii. A, Amino acid sequence alignment of plant Ga proteins representing major taxa was performed using ClustalW (www.clustal.org). Identical amino acid residues are highlighted in black, and blocks of amino acids with similar biochemical features are highlighted in gray. Black and gray letters represent conserved and nonsimilar amino acid residues, respectively. Consensus sequences for GTP binding and hydrolysis are labeled G1 to G5. The conserved Met and the invariant Gln, corresponding to positions 1 and 222 in AtGPA1, are labeled with asterisks. P/M represents the predicted sites for palmitoylation/myristoylation. B, The phylogenetic relationship of plant Ga proteins was inferred using the
expression of higher plant G-protein genes, which also show a low but ubiquitous expression (Bisht et al., 2011; Choudhury et al., 2011). The relative expression levels of CbGa, CbGb, CbGγ, and CbRGS were between 2% and 15% of the expression of the C. braunii ELONGATION FACTOR1α gene (CbEF1; GenBank accession no. AF032728.1), which was used as an internal control. In comparison, the level of 18S ribosomal RNA expression levels were measured as an internal control.

CbGa is an active GTP-binding protein and displays GTPase activity, as shown by a classic increase in fluorescence followed by a gradual decrease over time (Fig. 3A). The CbGa nucleotide binding is GTP specific, as indicated by using non-BODIPY-conjugated GTP, ATP, or ADP as a competitor to the labeled BODIPY-GTP-FL in binding assays. The addition of GTP, but not of other nucleotides, reduced the BODIPY-GTP-FL-specific fluorescence signal.

To evaluate whether the functional interaction between CbGa and CbRGS proteins is conserved, the GTPase activity of CbGa was assayed in the presence of the purified, recombinant RGS domain of C. braunii. The CbGa GTPase activity can be significantly accelerated by the presence of CbRGS (Fig. 3B). Interestingly, despite the relatively low sequence identity between the Arabidopsis and C. braunii RGS domains, their cross-species functionality is maintained. Purified recombinant AtRGS1 protein (RGS domain) accelerated the GTPase activity of CbGa to a similar extent as the CbRGS protein (Fig. 3B). Conversely, the CbRGS protein was able to accelerate the GTPase activity of AtGPA1 (Fig. 3C). This suggests that the critical residues required for the activity of these proteins are conserved from the date of divergence between Chara species and land plants.

The Interaction between Different C. braunii G-Protein Subunits and Its Regulator Is Conserved

A functional C. braunii G-protein complex necessitates the physical interaction between individual subunits as well as between Ga and RGS proteins. To ascertain such an interaction, targeted interaction analyses were performed between CbGa and CbGb and between CbGa and CbRGS, using the membrane-based split-ubiquitin assay, and between CbGb and CbGγ using the classic yeast (Saccharomyces cerevisiae) two-hybrid assay. CbGb and CbGγ subunits displayed strong and specific interaction when used as fusion constructs with the DNA activation or DNA-binding domains (Fig. 4A). Similarly, the interaction between the full-length CbGa and CbGb proteins as well as the interaction of CbGa and the RGS domain of CbRGS could be verified in split-ubiquitin assays (Fig. 4B). CbGa and the RGS proteins interacted in all of the tested combinations. However, CbGa and CbGb proteins interacted only in specific combinations of different split-ubiquitin fusions. CbGa interacted with CbGb only when the Nub fusion was made at its...
C terminus (Ga::Nub + Gb::Cub) or when the Ga protein was used as a Cub fusion protein (Ga::Cub + Nub::Gb). This was expected, as the N terminus of Ga proteins is required for their interaction with the Gb proteins, and fusion of the ubiquitin half at the N terminus of the Ga protein might interfere with this interaction (Cabrera-Vera et al., 2003). When CbGb was used as a Nub fusion protein, interaction was detected only in the Nub-Gb orientation (Nub::Gb + Ga::Cub). It is well known that the Cub fusion constructs have a significantly higher affinity for the Nub-X constructs than for the X-Nub constructs. As a result, weaker interactions between two proteins may not be detected when the test protein is in the X-Nub orientation, as has

Figure 3. Activity assay of CbGa and CbRGS proteins. A, GTP binding and hydrolysis of recombinant, purified CbGa were measured using BODIPY-FL in real-time fluorescence-based assays. The specificity of GTP binding was tested by adding nonfluorescent GTP, ATP, or ADP in the reaction mix, where only nonfluorescent GTP was able to compete efficiently for fluorescent GTP binding. B, GTPase activity of CbGa is accelerated by recombinant, purified RGS domains of CbRGS or AtRGS1 proteins in BODIPY-FL-based real-time fluorescence assays. C, GTPase activity of AtGPA1 is accelerated by recombinant, purified RGS domains of AtRGS1 or CbRGS. All data are from one of three independent experiments, each with three replicates (means ± SD).

Figure 4. Physical interactions among C. braunii G-protein components. A, Protein-protein interaction between CbGb and CbGy was demonstrated using yeast two-hybrid-based growth and colorimetric assays. Physical interaction between CbGb and CbGy could be demonstrated in either fusion construct combination as DNA activation domain (AD) or DNA-binding domain (DBD) fusions. B, Interaction between CbGa and CbGb as well as between CbGa and the RGS domain of CbRGS was tested in a split-ubiquitin-based system. CbGa and CbRGS interacted with each other in all orientations (N-terminal and C-terminal fusions with the N terminus of ubiquitin [Nub::X, X::Nub] as well as when fused with the C terminus of ubiquitin [Y::Cub]). CbGa interacted with CbGb only when used as a Cub fusion protein or as a CbGa::Nub fusion, whereas Nub::CbGb but not CbGb::Nub interacted with CbGa::Cub. Interactions were tested by 5-bromo-4-chloro-3-indolyl β-d-galactopyranoside (X-Gal) filter-lift assays, and Nub vector fusions with empty Cub vectors were used as negative controls. SC-LT, media lacking Leu, Trp. [See online article for color version of this figure.]
also been shown for the interaction of the KATI protein (Obrdlik et al., 2004). Nonetheless, the interaction data suggest the existence of both the trimeric and dimeric conformations of specific G-protein subunits in Chara species, which is consistent with the classical G-protein signaling mechanism.

Taken together, these data firmly establish that the green algae of the order Charales, which are close relatives to land plants, possess a complete functional repertoire of G-proteins. It is evident, therefore, that G-protein signaling in the green lineage was established before embryophytes evolved and plants started occupying terrestrial habitats.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Chara braunii cultures (kindly provided by Dr. Ilse Foisser) were cultivated in distilled water on a sand/soil/peat mixture at room temperature and a 14/10-h light/dark cycle as described previously (Schmölzer et al., 2011).

G-Protein Gene Identification

C. braunii G-protein sequences were aligned by BLAST analysis of a single lane of a transcriptome data set with Arabidopsis (Arabidopsis thaliana) G-protein sequences as queries. The RNA-Seq library was made using C. braunii thalli at the vegetative growth phase. The transcriptome was sequenced using the Illumina HiSEQ2000 platform, and the sequence reads were assembled using ABySS (http://www.bcgsc.ca/platform/bioinfo/software/abyss; Abe et al., 2012). Full-length G-protein genes were amplified from C. braunii cDNA using gene-specific primers (Supplemental Table S2). All G-protein genes were cloned into pCR8/GW/TOPO vector (Life Technologies) and confirmed by sequencing.

Phylogenetic Analyses

Go- and RGS-related genes were aligned using Clustal Omega (Sievers et al., 2011). Phylogenetic analyses were conducted by the maximum likelihood method under the JTT matrix-based model (Jones et al., 1992) using MEGA5 (Tamura et al., 2011). Initial trees for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using a JTT model. All positions containing gaps and missing data were eliminated.

RNA Isolation and Quantitative Expression Analysis

Total RNA was isolated with the RNeasy Plant Mini Kit (Qiagen) according to the manufacturer’s protocol using 100 mg of C. braunii internodal or dimorphic branch tissue. Genomic DNA was removed from total RNA by using Turbo DNA-free. One microgram of RNA was used for first-strand synthesis using SuperScript III reverse transcriptase (Life Technologies) along with 50 ng of random hexamer oligonucleotides. Quantitative reverse transcription-PCR experiments were performed in 20-μL reactions (2 μL of 1:10 diluted cDNA, 200 nM each primer, 0.4 μL of ROX Reference Dye, and 10 μL of 2× SYBR Advantage qPCR Premix; Clontech) with the StepOnePlus Real-Time PCR System. Amplicon integrity was verified by the analysis of melting curves and agarose gel electrophoresis. Primer efficiency was determined by linear regression on the log (fluorescence) of each PCR using the LinRegPCR software (Ramakers et al., 2003), and transcript levels of each gene were normalized to CHEFI (GenBank accession no. AF032728.1).

Recombinant Protein Purification and G-Protein Activity Assay

Full-length CbGs and AtGPA1 as well as RGS domains of CbRGS (amino acids 401–406) and AtRGS1 (amino acids 250–459) were cloned into the pET-28a vector (Novagen) and expressed in Escherichia coli strain BL21 (DE3). Recombinant protein purification and real-time fluorescence-based GTP binding and GTP hydrolysis assays were performed as described previously (Choudhury et al., 2013).

Protein-Protein Interaction Assays

The interaction assays between CbGs and CbGβ and between CbGs and CbRGS were performed using the mating-based yeast split-ubiquitin system (Obrdlik et al., 2004; Pandey and Assmann, 2004). Protein-protein interactions were determined by the growth of diploid yeast colonies on minimal medium lacking Leu, Trp, His, and adenine but containing 200 μM Met. Interactions were confirmed by filter-lift assays using 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside as a substrate (Schneider et al., 1966). The interaction between CbGβ and CbGγ was determined using a Gateway-based yeast two-hybrid assay (ProQuest Two Hybrid System; Life Technologies) as described previously (Choudhury et al., 2011). Yeasts, cotransformed with the gene of interest and an empty vector, were used as negative controls. Interactions between proteins were identified by growth on medium lacking Leu, Trp, His, and uracil as well as by filter-lift assays using 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside as a substrate.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers KF781935 (CbGα), KF781957 (CbGβ), KF781958 (CbGγ), and KF781959 (CbRGS).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Amino acid sequence alignment of Gβ proteins of different plant species representing major taxa.

Supplemental Figure S2. Amino acid sequence alignment of Gγ proteins of different plant species representing major taxa.

Supplemental Figure S3. Amino acid sequence alignment of RGS proteins of different plant species representing major taxa.

Supplemental Table S1. Percent identity matrix of selected plant Gα proteins generated with Clustal W2.1.

Supplemental Table S2. Primer sequences for gene-specific quantitative PCR analysis.

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LITERATURE CITED


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