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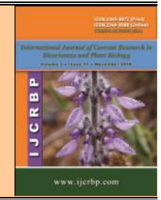

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Prediction of G Protein Coupled Receptors from Plant Genomes

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Abstract

G-protein coupled receptors (GPCRs) are the largest family of membrane proteins, as key components of signal transduction pathways. Despite the current knowledge, GPCRs in plants are not well characterized if compared with animals and are still a matter of controversy. The availability of plant genomes allows the prediction and characterization of diverse GPCRs, as for several species the repertoire of GPCRs remains unclear. As a contribution to this problem, we present the *in silico* analyses of putative GPCRs candidates from *Arabidopsis thaliana, Capsicum annuum, Glycine max, Manihot esculenta, Medicago truncatula, Oryza sativa, Phaseolus vulgaris, Populus trichocarpa, Ricinus communis, Solanum lycopersicum, Solanum tuberosum, Sorghum bicolor, Theobroma cacao, Triticum aestivum, Vitis vinifera* and *Zea mays* proteomes. From a total of 997,435 proteins collected, the sequences were selected by length, reducing the initial collection to 487,012. GPCRpipe program predicted 60 GPCRs candidates, ranging from none (*Capsicum annuum*) to 12 candidates (*Populus trichocarpa)* (median = 3 ± 2.90).The Neighbor joining dendrogram of the 60 predicted GPCR candidates grouped the proteins in three clades. These findings may indicate that GPCR in plants are involved in drought stress responses.

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Keywords

G protein coupled receptors
Membrane protein
Protein prediction
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Introduction

G-protein coupled receptors (GPCRs) belong to the largest family of membrane proteins as key components of signal transduction pathways. They are activated by diverse ligands, including odorants, fatty acids, peptides and neurotransmitters, across cell membranes. GPCRs are responsible for the communication between cell and environment through GPCRs signaling. Although GPCRs are important to pharmaceutical application, very few is known about plant GPCRs. Usually, the identification of novel GPCRs from genome analysis allowed the prediction of new important receptors in several species, but GPCRs in plants are still a matter of debate if compared to animal GPCRs (Assmann, 2002; Trusov and Botella, 2016; Yadav and Tuteja, 2011). Most of the controversy about the existence of GPCRs in plants is due to the functional paradigm of animal kingdom, where the action of GPCRs allowed the development of complex signaling and sensing processes, yet different in plants (Tadesse et al, 2014).

Compared to the animal kingdom where thousands of GPCRs were identified, only a single GPCR has been yet clearly identified in plant system: in *Arabidopsis*, the gene corresponding to the canonical GPCR candidate was isolated by Josefsson and Rask (1997) and Plakidou-Dymock et al. (1998) which concluded that GCR1 encodes the first 7TM receptor homologue identified in higher plants and is involved in cytokinin signal transduction. In the last decade, the physiological effect of GCR1 has been investigated and several studies put together information about the seed germination, the interaction with cell signaling mediators, add to the response to brassinosteroids and gibberellins, as well as reducing drought stress (Chakraborty et al., 2015b). The genome mining of *Arabidopsis* described a computational strategy that identified 54 7TMpR candidates but only one as GCR1 (Moriyama et al., 2006). Recently, Chakraborty et al. (2015a) provided experimental validation for the physiological role of GCR1 in which an extensive genome wide response of unknown genes and processes has been suggested. Moreover, the authors pointed to the need of continue studying the role of GCR1 in cell signaling in *Arabidopsis*, including considering the contest to write-off plant GPCRs, or their role in plant G-protein signaling. From the three putative GPCRs in *Arabidopsis* only one, GCR1, shows marked homology with GPCRs (Assmann, 2002). From *Dictyostelium*, one cAMP receptor CAR1 has 20 to 23% identity with the CARs. The Medicago RGS-protein homolog shares 60% sequence identity (Temple and Jones, 2007).

The availability of several plant genomes made possible the prediction and characterization of diverse GPCRs as well as a comparative analysis of the GPCR structure-function relationship in plants. Gookin and coworkers (2008) performed a whole proteome survey for GPCRs in three plant genomes, namely *Arabidopsis*, poplar and rice. The authors used experiments binding tests with GPA1 and showed through G-protein coupled receptor classification and molecular evolutionary analyses that both individual G-protein coupled receptor candidates and candidate G-protein coupled receptor families are conserved among plant species. We have extended this work and in the present paper we describe the comparative survey for GPCRs in sixteen plant genomes. We expect that this paper will contribute to solve this debate and increase knowledge in plant GPCR candidates.

Materials and methods

The sixteen proteomes used for detecting GPCRs were downloaded from several database repositories, as described on Table 1.

Table 1. Dataset sources and versions used for GPCR detection.

Organism	Source	Version (Reference)
Arabidopsis thaliana	TAIR: The *Arabidopsis* Information Resource	10.29 (Lamesch et al., 2012)
Capsicum annuum	Solgenomics	1.55 (Kim et al., 2014)
Glycine max	Phytozome	1.1 (Schmutz et al., 2010)
Manihot esculenta	Phytozome	6.1 (Prochnik et al., 2012)
Oryza sativa	Phytozome	7.0 (Ouyang et al., 2007)
Phaseolus vulgaris	Phytozome	1.0 (Schmutz et al., 2014)
Solanum lycopersicum	Solgenomics	2.4 (Sato et al., 2012)
Solanum tuberosum	Solgenomics	3.4 (Xu et al., 2011)
Triticum aestivum	Phytozome	2.2 (Mayer et al., 2014)
Zea mays	EnsemblPlant	AGPv3.26 (Schnable et al., 2009)
Vitis vinifera	Phytozome	Genoscope.12X (Jaillon et al., 2007)
Theobroma cacao	Phytozome	1.1 (Motamayor et al., 2013)
Sorghum bicolor	Phytozome	3.1 (Not published)
Ricinus communis	Phytozome	0.1 (Chan et al., 2010)
Populus trichocarpa	Phytozome	3.0 (Tuskan et al., 2006)
Medicago truncatula	Phytozome	4.0 (Young et al., 2011)

GPCR identification

To identify candidate GPCRs we performed a bioinformatic analyses concatenated as described in Fig. 1 where several softwares were used to predict protein topology and to classify putative GPCRs. Each proteome set was analyzed independently. The first step consisted to filter all the proteins according to their size. As the large majority of GPCRs contains between 250 and 1000 residues, the selected proteins by our pipeline were the ones within this range. The second step was to submit the selected proteins to the

Transmembrane helix predictors in order to identify proteins with TM regions (Krogh et al., 2001). The third step was the prediction of GPCRs through GPCRpipe program (Theodoropoulou et al., 2013). Two methods in GPCR pipe were used for the detection of GPCR: 1) Based on Hidden Markov Model, where the first step was designed for the detection of GPCR. 2) Using a library of domains consisting in 39 Pfam profile HMMs (specific to different families of GPCR). We used the GPCRpipe "AND" method, meaning that a GPCR was predicted only if there was a consensus between the two methods to confirm the prediction. This choice resulted in a small number of GPCR candidates but, on the other hand, limited the number of false positive predictions. The final step was the validation of GPCRpipe candidate predictions, through the protein domain prediction with ProDom (Corpet et al., 2000). Only proteins annotated with a GPCR domain were finally considered as putative GPCR candidate.

Transmembrane helix predictions

Five programs were used to predict transmembrane helices. Topology prediction was performed on each selected proteome by analyzing the complete sequences with TMHMM version 2.0 (Krogh et al., 2001), Phobius (Käll et al., 2007), HMMTOP2 (Tusnády and Simon, 2001), MemBrain (Shen and Chou, 2008) and Octopus (Viklund and Elofsson, 2008).

GPCR classification

The candidate proteins were grouped based on the sequence similarity and class recognition through CLUSTALW alignment (Higgins and Sharp, 1998). The phylogenetic tree of the GPCR candidates was built by Mega 6.0 using Neighbor-joining method and 10,000 repetitions. In parallel, the sequence of each GPCR candidate was submitted to two web servers: PCA-GPCR (Peng et al., 2010) and GPCRPred (Bhasin and Raghava, 2004) dedicated to GPCR class recognition. Each of these prediction methods provides the GPCR class and its subfamily. The transmembrane helices were aligned using TM-Coffee (Chang et al., 2012).

Gene ontology and domain identification

The functional analysis (GO) of the proteins was predicted using AgBase 2.0, a curated resource for agricultural plant gene products (McCarthy et al., 2011). For GO annotation, the protein sequences were submitted to Goanna, using as database the AgBase-UniProt. All the evidence codes were selected and the others parameters were set as standard. The summarized results retrieved from Goanna (slim input) were submitted to GoSlim Viewer (McCarthy et al., 2006). The redundant GO terms were removed by REVIGO (Supek et al., 2011). For domain identification, the sequences were submitted to InterProScan (Jones et al., 2014). The prediction of the presence and location of signal peptide cleavage sites in amino acid sequences was done with SignalP (SignalP 4.0 - Discrimination between Signal Peptides and Transmembrane Regions).

Results

In order to explore the GPCR repertoire in plant genomes, we selected data from different sources and taxonomic families, i.e., Solanaceae, Malvaceae, Oryzeae, Triticeae, Fabaceae, Vitaceae, Euphorbiaceae, Flacourtiaceae and Poaceae. Following the pipeline described in Fig. 1, the 997,435 proteins from the sixteen selected plants were filtered by length (proteins between 250 and 1000 amino acids) resulting in 487,012 candidates. To reduce the number of proteins to be submitted to GPCRpipe, only proteins with seven transmembrane helices (7TM) were retained. This resulted in 4,878 proteins which were submitted to the GPCRpipe program. The bioinformatic analysis predicted a total amount of 60 GPCRs (Tables 2 and 3). The number of candidates reflects the stringency of the methods, as for *Arabidopsis*, p.e., our system predicted the 5 putative GPCRs, already known (Yadav and Tuteja, 2011). For Populus, Medicago and rice the results were lower than predicted previously by Gookin et al. (2008), Chen and Ellis (2008) and Taddese et al. (2014). While for other species the number of GPCR candidates is a novelty.

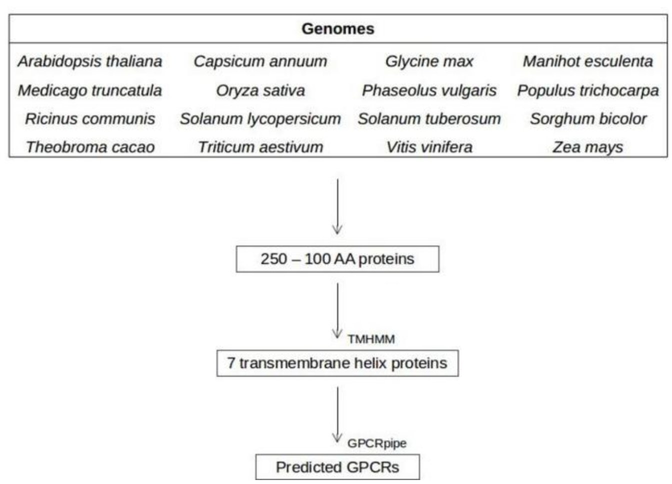


Fig. 1: Data analysis framework for prediction of GPCRs in plant genomes.

Table 2. Number of proteins and GPCR candidates for each plant species genome selected.

Species	Proteome	# 250-1000 AA proteins	# 7 transmembrane helix proteins	# GPCR Candidates
Arabidopsis thaliana	35,386	22,426	198	5
Capsicum annuum	34,900	7,086	57	0
Glycine max	73,320	43,970	423	5
Manihot esculenta	41,381	24,864	260	2
Medicago truncatula	62,319	30,855	268	3
Oryza sativa	49,061	26,790	236	1
Phaseolus vulgaris	31,638	20,386	220	3
Populus trichocarpa	73,013	44,539	444	12
Ricinus communis	31,221	15,171	140	2
Solanum lypersicum	34,725	2,166	30	1
Solanum tuberosum	56,228	29,931	242	4
Sorghum bicolor	47,205	28,695	283	4
Theobroma cacao	44,404	27,627	252	3
Triticum aestivum	293,053	129,620	1,383	8
Vitis vinifera	26,346	13.653	128	4
Zea mays	63,235	32,872	314	3

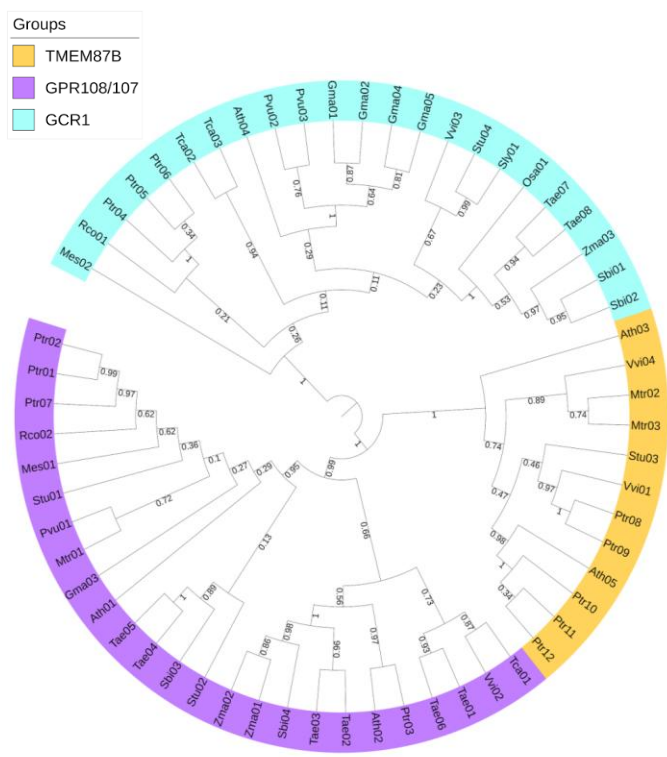


Fig. 2: Neighbor joining dendrogram of the 60 predicted GPCR candidates. Bootstrap values (10,000 replications) are written on the branches. Color-code corresponds to GPCR Classes.

The Neighbor joining dendrogram of the 60 predicted GPCR candidates grouped the proteins in three clades, corresponding to domains G protein-coupled receptor 1 (GCR1), G protein-coupled receptor 108 (GPR108) and Transmembrane protein 87B (TMEM87b) (Figs. 2 and 3). The domains GPR108 and TMEM87b are members of subfamilies A and B, respectively, of the Lung seven transmembrane (LUSTR) family. They are found in plants, animals and in fungi (Edgar, 2007). Sequence comparison of the 60 candidates is shown in Fig. 4.

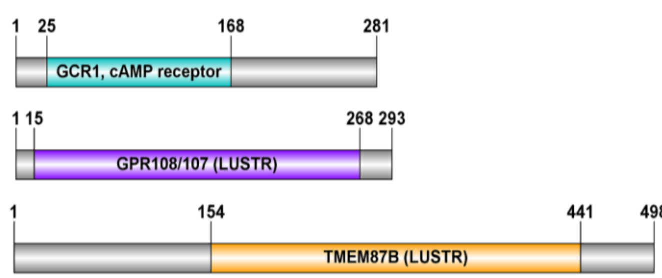
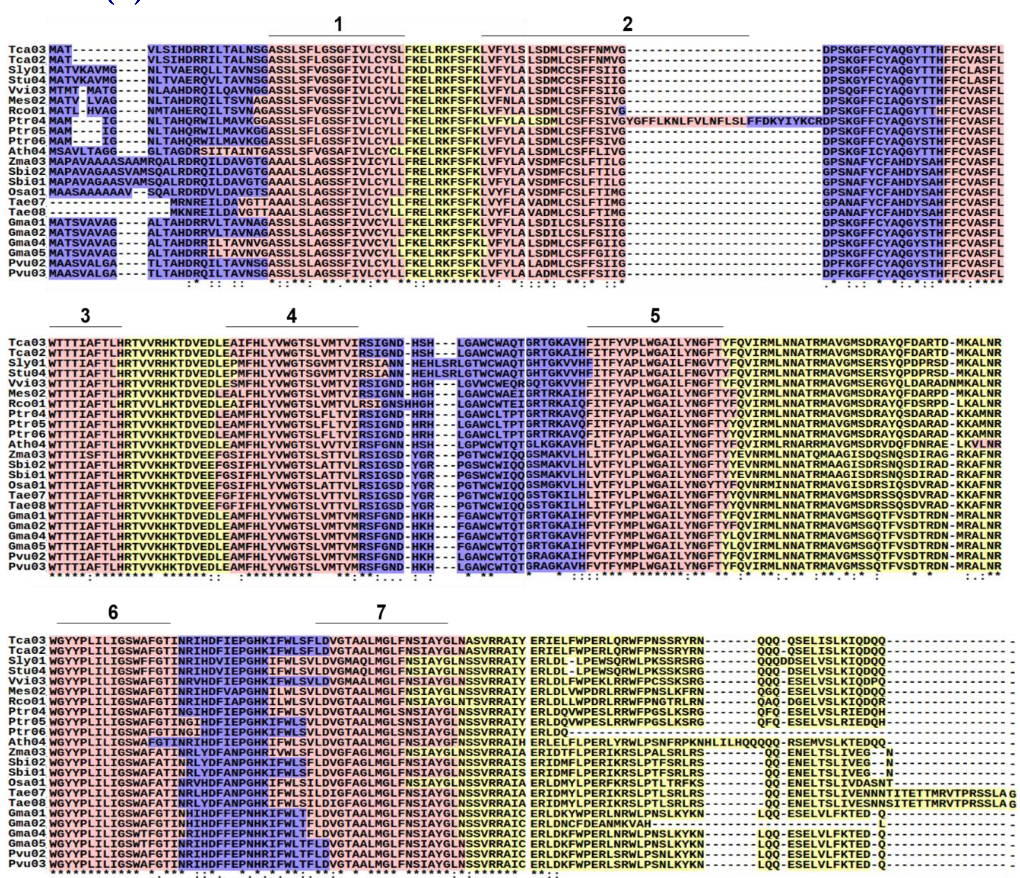


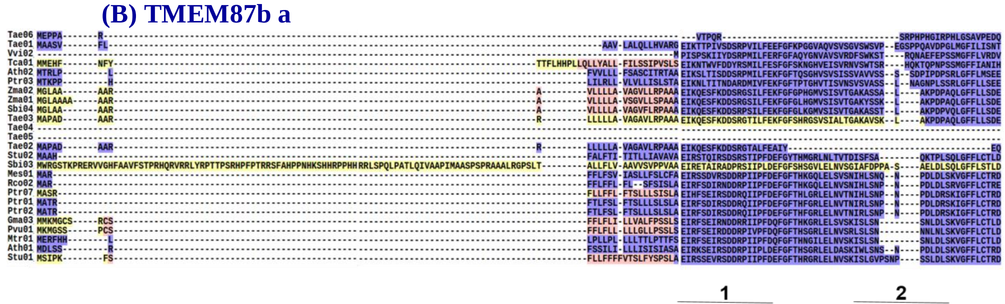
Fig. 3: Structure of conserved domains of putative Plant GPCRs. GCR1, cAMP receptor - G protein-coupled receptor 1 cAMP receptor; GPR108 -G protein-coupled receptor 108 and TMEM87b- Transmembrane protein 87B. The domains GPR108 and TMEM87b are members of subfamilies A and B, respectively, of the Lung seven transmembrane (LUSTR) family.

The clustering in three groups corresponding to the three domains reflect the protein sequence alignment where the GCR1 group show higher conservation in the TM helices segments as well as in the loops, except for the candidate Ptr04 from *P. trichocarpa* that show an insertion in the sequence. All members of these groups have no signal peptide. The GPR108 group, on the other hand, shows more conservation only on C-terminal and more diversity on the N-terminal segment, corresponding to the extracellular topology. The majority of the sequences present signal peptide. The TMEM87b group shows high similarity on the core of the protein, corresponding to the topology of transmembrane segments but lack of similarity is observed either on N-terminal as well as in the C-terminal. All members of this groups present signal peptide (Table 3). Table 4 describes the Gene Ontology prediction for each clade, where the group corresponding to GCR1 domain, with 23 protein candidates, is associated with the biological process related to abscisic acid-activated signaling pathway, in the membrane with signal transducer activity. These findings suggest the conservation of GCR1 among plants from different taxonomic families, both monocots and dicots. Among the 60 candidates, 25 are indicated to participate on root morphogenesis and most the 12 candidates like the TMEM87b have no annotation described by Gene Ontology.

(A) GCR1



(B) TMEM87b a



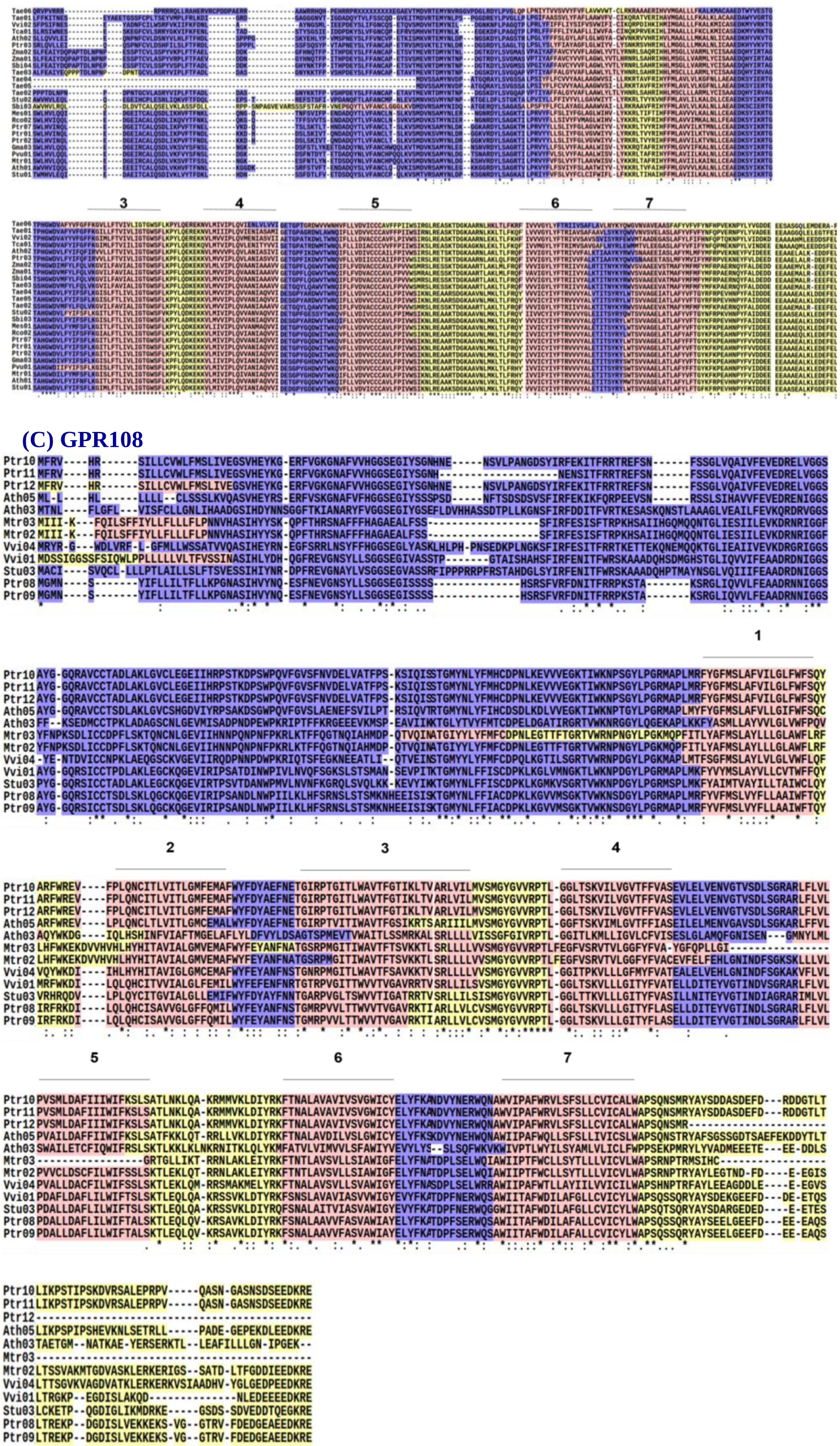


Fig. 4: Multiple sequence alignment for the transmembrane domain regions of the putative Plant GPCRs. Yellow: Intracellular loops, Pink: transmembrane helices, Blue: Extracellular loops. (A) GCR1, (B) TMEM87b a (C) GPR108.

Table 3. Dataset sources and versions used for GPCR detection.

GPCR candidate			Blast Results			TMHMM prediction	Signal P prediction	Group	Class prediction
Id	Seq Name	Species	Accession	Description	Species				
Ath01	AT5G42090.1	*Arabidopsis thaliana*	NM_123574	lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Ath02	AT3G09570.1	*Arabidopsis thaliana*	NM_111791	lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Ath03	AT1G10980.1	*Arabidopsis thaliana*	NM_100973	Lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Ath04	AT1G48270.1_(GCR1)	*Arabidopsis thaliana*	NM_103724	G-protein-coupled receptor 1	*Arabidopsis thaliana*	7	No	Gr 1	Amine Subfamily of CLASS A
Ath05	AT1G72480.1	*Arabidopsis thaliana*	NM_105907	Lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Gma01	Glyma14g12700.2	*Glycine max*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Not a GPCR
Gma02	Glyma14g12700.3	*Glycine max*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Not a GPCR
Gma03	Glyma02g09690.1	*Glycine max*	XM_013605659	lung seven transmembrane receptor family protein	*Medicago truncatula*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Gma04	Glyma17g33480.1	*Glycine max*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Not a GPCR
Gma05	Glyma17g33480.2	*Glycine max*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Not a GPCR
Mes01	Manes.02G001900.1.p	*Manihot esculenta*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Mes02	Manes.03G212200.1.p	*Manihot esculenta*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Mtr01	Medtr3g080830.1	*Medicago truncatula*	XM_013605659	lung seven transmembrane receptor family protein	*Medicago truncatula*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Mtr02	Medtr8g005790.1	*Medicago truncatula*	XM_003626631	lung seven transmembrane receptor family protein	*Medicago truncatula*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Mtr03	Medtr8g005485.1	*Medicago truncatula*	XM_013588310	lung seven transmembrane receptor family protein	*Medicago truncatula*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Osa01	LOC_Os06g09930.1	*Oryza sativa*	NM_001153424	G protein-coupled receptor	*Zea mays*	7	No	Gr 1	Not a GPCR
Ptr01	Potri.003G147700.2	*Populus trichocarpa*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Ptr02	Potri.003G147700.1	*Populus trichocarpa*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Ptr03	Potri.006G214900.1	*Populus trichocarpa*	XM_007027081	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Ptr04	Potri.008G206000.1	*Populus trichocarpa*	XM_002311815	G-protein-COUPLED RECEPTOR 1	*Populus trichocarpa*	7	No	Gr 1	Amine Subfamily of CLASS A
Ptr05	Potri.008G206000.2	*Populus trichocarpa*	XM_002311815	G-protein-COUPLED RECEPTOR 1	*Populus trichocarpa*	7	No	Gr 1	Amine Subfamily of CLASS A
Ptr06	Potri.008G206000.3	*Populus trichocarpa*	XM_002311815	G-protein-COUPLED RECEPTOR 1	*Populus trichocarpa*	7	No	Gr 1	Not a GPCR
Ptr07	Potri.001G083100.1	*Populus trichocarpa*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Ptr08	Potri.001G209400.2	*Populus trichocarpa*	XM_007041491	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A

GPCR candidate			Blast Results			TMHMM prediction	Signal P prediction	Group	Class prediction
Id	**Seq Name**	**Species**	**Accession**	**Description**	**Species**				
Ptr09	Potri.001G209400.1	*Populus trichocarpa*	XM_007041491	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Ptr10	Potri.001G166500.1	*Populus trichocarpa*	XM_007024608	Lung seven transmembrane receptor family protein isoform 1	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Ptr11	Potri.001G166500.2	*Populus trichocarpa*	XM_007024608	Lung seven transmembrane receptor family protein isoform 1	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Ptr12	Potri.001G166500.3	*Populus trichocarpa*	XM_007024608	Lung seven transmembrane receptor family protein isoform 1	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Pvu01	Phvul.004G090500.1	*Phaseolus vulgaris*	XM_013605659	lung seven transmembrane receptor family protein	*Medicago truncatula*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Pvu02	Phvul.001G044800.1	*Phaseolus vulgaris*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Pvu03	Phvul.001G044800.2	*Phaseolus vulgaris*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Rco01	29889.m003405	*Ricinus communis*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Rco02	30068.m002586	*Ricinus communis*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Sbi01	Sobic.010G074900.1.p	*Sorghum bicolor*	NM_001153424	G protein-coupled receptor	*Zea mays*	7	No	Gr 1	Amine Subfamily of CLASS A
Sbi02	Sobic.010G074900.2.p	*Sorghum bicolor*	NM_001153424	G protein-coupled receptor	*Zea mays*	7	No	Gr 1	Amine Subfamily of CLASS A
Sbi03	Sobic.010G022300.2.p	*Sorghum bicolor*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	No	Gr 2	Amine Subfamily of CLASS A
Sbi04	Sobic.003G348200.1.p	*Sorghum bicolor*	NM_121857	candidate G-protein Coupled Receptor 7	*Arabidopsis thaliana*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Sly01	Solyc08g061260.2.1	*Solanum lycopersicum*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Stu01	PGSC0003DMP400010386	*Solanum tuberosum*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Stu02	PGSC0003DMP400052623	*Solanum tuberosum*	XM_007039313	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Stu03	PGSC0003DMP400031132	*Solanum tuberosum*	XM_007041491	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Stu04	PGSC0003DMP400023027	*Solanum tuberosum*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	GR 1	Amine Subfamily of CLASS A
Tae01	Traes_2BL_E9C46CB57.1	*Triticum aestivum*	NM_001158876	protein GPR106	*Zea mays*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Tae02	Traes_3B_E2D1FD54F.1	*Triticum aestivum*	NM_111791	lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Tae03	Traes_3B_E2D1FD54F.2	*Triticum aestivum*	NM_111791	lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Tae04	Traes_4AL_F4A73435D.1	*Triticum aestivum*	NM_123574	lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	No	Gr 2	Amine Subfamily of CLASS A
Tae05	Traes_4AL_F4A73435D.2	*Triticum aestivum*	NM_123574	lung seven transmembrane receptor family protein	*Arabidopsis thaliana*	7	No	Gr 2	Amine Subfamily of CLASS A
Tae06	Traes_6AL_3320984F2.1	*Triticum aestivum*	NM_001158273	protein GPR107	*Zea mays*	7	No	Gr 2	Amine Subfamily of CLASS A

GPCR candidate			Blast Results			TMHMM prediction	Signal P prediction	Group	Class prediction
Id	Seq Name	Species	Accession	Description	Species				
Tae07	Traes_7AS_57E069383.1	*Triticum aestivum*	NM_001153424	G protein-coupled receptor	*Zea mays*	7	No	Gr 1	Amine Subfamily of CLASS A
Tae08	Traes_7DS_653DD38AF.1	*Triticum aestivum*	NM_001153424	G protein-coupled receptor	*Zea mays*	7	No	Gr 1	Amine Subfamily of CLASS A
Tca01	Thecc1EG026130t1	*Theobroma cacao*	NM_001158876	Lung seven transmembrane receptor, putative	*Theobroma cacao*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Tca02	Thecc1EG021504t1	*Theobroma cacao*	XM_007035944	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Tca03	Thecc1EG021504t2	*Theobroma cacao*	XM_007035945	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Vvi01	GSVIVT01023009001	*Vitis vinifera*	XM_007041491	Lung seven transmembrane receptor family protein	*Theobroma cacao*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Vvi02	GSVIVT01026321001	*Vitis vinifera*	NM_001158876	Lung seven transmembrane receptor, putative	*Theobroma cacao*	7	No	Gr 2	Amine Subfamily of CLASS A
Vvi03	GSVIVT01035487001	*Vitis vinifera*	XM_007035945	G-protein-coupled receptor 1 isoform 1	*Theobroma cacao*	7	No	Gr 1	Amine Subfamily of CLASS A
Vvi04	GSVIVT01032664001	*Vitis vinifera*	XM_003615364	lung seven transmembrane receptor family protein	*Medicago truncatula*	7	Yes	Gr 3	Amine Subfamily of CLASS A
Zma01	AC214448.3_FGP007	*Zea mays*	NM_001157220	transmembrane receptor	*Zea mays*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Zma02	GRMZM2G179985_P01	*Zea mays*	NM_121857	candidate G-protein Coupled Receptor 8	*Arabidopsis thaliana*	7	Yes	Gr 2	Amine Subfamily of CLASS A
Zma03	GRMZM2G120371_P01	*Zea mays*	NM_001153424	G protein-coupled receptor	*Zea mays*	7	No	Gr 1	Not a GPCR

Table 4. Gene ontology analysis ofthe GPCR candidates.

Domains	Biological process annotation	Cellular component annotation	Molecular function annotation
GCR 1	Abscisic acid-activated signaling pathway Activation of phospholipase C activity Cell cycle G-protein coupled receptor signaling pathway Lipid metabolic process Maintenance of seed dormancy Mitotic cell cycle Regulation of inositol trisphosphate biosynthetic process Response to low fluence blue light stimulus Tyrosine biosynthetic process	Golgi apparatus Integral component of membrane Membrane Plasma membrane Spanning component of plasma membrane Vacuolar membrane	G-protein coupled receptor activity protein binding Signal transducer activity Transmembrane signaling receptor activity
GPR107/108 (LUSTR)	Response to molecule of bacterial origin Root morphogenesis	Integral component of membrane Membrane Trans-Golgi network	No annotation
TMEM87B (LUSTR)	No annotation	No annotation	No annotation

Discussion

Genomic sequencing has provided a vast resource for identifying interesting genes, but the discovery of new GPCR in plant genomes is not straightforward. The proteins often show low sequence similarities and there is a huge lack of experimental information about plant GPCRs structure-function relationship (Taddese et al., 2014). The usual protein sequence search is not able to detect new GPCRs candidates, although sharing similar structural topology (Zhang, 2012) as GPCRs vary dramatically in development of logic survey and prediction of candidates through bioinformatics studies, before their experimental validation during the past two decades (Yarnitzky et al., 2010). This matter remains under constant review and development (Esguerra et al., 2016; Lu and Wu, 2016; Zhang, 2012).

According to Urano and Jones (2014), plant G proteins spontaneously release GDP and bind GTP *in vitro*, and thus are self-activating and in contrast to animal mechanisms, the presence of a signal inhibits deactivation of constitutive G activation. The authors conclude that plants do not need classic GPCRs although there are approximately 50 proteins in *Arabidopsis* and rice that potentially have the same topology as human GPCRs. The lack of experimental elucidation increased the controversy (Coopman et al., 2011; Taddese et al., 2014).

Josefsson and Rask (1997)and Plakidou-Dymock et al. (1998) firstly reported the discovery of a putative classic GPCR in *Arabidopsis* which concluded that GCR1 encodes the first 7TM receptor homologue identified and was involved in cytokinin signal transduction (Assmann, 2002). Increasing the knowledge on this field, in the last decade, the physiological effect of GCR1 has been investigated and several studies put together information about the seed germination, the interaction with cell signaling mediators, add to the response to brassinosteroids and gibberellins, as well as reducing drought stress (Chakraborty et al., 2015b; Moriyama et al., 2006). Central to this analysis is the prediction of conserved, hydrophobic, seven transmembrane (7-TM) domain that locates the GPCR and dictates its minimum amino acid sequence length (~260 amino acids) (Davies et al., 2007), despite the low sequence similarity is observed when comparing sequences, usually around 25%.

Thus, to predict GPCRs, the search for topology patterns and the identification of seven hydrophobic regions is one important step (Urano and Jones, 2013). It is, therefore, necessary to consider fold and motifs (Krishnan et al., 2012) to distinguish between GPCRs and other proteins that may share a 7TM scaffold. The observation of conserved domains in GPCR candidates enables an integrated classification and clustering based on secondary structure (Gookin et al., 2008). Our analysis identified signatures involving the role of conserved residues such as the GCR1 domain and the GPR108 and TMEM87b from the Lung seven transmembrane (LUSTR) domain family in the three grouped candidates (Fig. 3 and 4). The LUSTR family has similar predicted topology and weak homology to bovine rhodopsin, a model GPCR. A gene duplication in an ancestral eukaryote has led to the evolution of two subfamilies (LUSTR-A and LUSTR-B) that have diverged significantly, whilst retaining close homology in the LUSTR domain within each subfamily (Edgar, 2007). In each LUSTR subfamily, the third intracellular loop is highly conserved, but distinct from each other. Generally, in other GPCRs, this third intracellular loop is involved in G-protein binding and receptor signaling suggesting that this region of the LUSTR proteins is also involved in G-protein- coupling. Studies in plants support the LUSTR involvement in an abiotic stress response. Cold stressed rice plants differentially express 123 transcripts, including a LUSTR homolog in the leaf tissue (Kim et al., 2007). The function associated to these proteins is still unknown, but high degree of sequence conservation suggests that these genes have retained an important function over long periods of eukaryotic evolution.

Moreover, Cvicek et al. (2016) recently described a list of conserved contact residues and important activation residues that are critical for GPCR folding and function. The authors showed that variants of amino acids at these locations in the GPCRs can alter the activation state and consequently change the cell signaling process. The conserved contacts appear to be conserved inside a class of GPCR structural fold. Furthermore, the authors propose that some residues identify structurally and functionally plant GPCRs candidates (Cvicek et al., 2016; Gookin et al., 2008).

Colaneri and Jones (2014) showed recently that plant G-proteins reach steady state at different active/inactive ratios under certain stresses (e.g. salt stress) the level of active Gα subunit and Gβγ is controlled by the energy state of the cell, which is sensed by the glucose level, and transduced by AtRGS1. Therefore, we speculate that new GPCR candidates are mainly related to GCR1 from the classical point of view, nevertheless the presence of others receptors and allow an integrative response to stimuli.

GPCRs and drought stress and signaling

Once perceived by a GPCR the external signal is transmitted into the cytoplasm by interaction with various downstream effectors proteins, providing a ubiquitous signaling mechanism in eukaryotes (Trusov and Botella, 2016). The phytohormone abscisic acid (ABA) plays a central role in plant development and in plant adaptation to both biotic and abiotic stressors. In the past years, much progress has been made on the identification of ABA receptors and the understanding of the molecular and biochemical mechanisms regarding ABA perception and transduction (Guo et al., 2011). Some studies have identified the GPCR-machinery as ABA receptors, located in various subcellular locations (Liu et al., 2007; Ma et al., 2009; Pandey et al., 2009; Park et al., 2009; Shen et al., 2006).

Under osmotic stress, ABA promotes plant water conservation by decreasing the apertures of stomatal pores in the epidermis. The role of GCR1 in Arabidopsis was investigated by a whole transcriptome microarray analysis of the GCR1-knock-out mutant and revealed that it has an extensive genome wide response. Since GCR1 and G-Protein A1 (GPA1) are physically coupled, it seems that GCR1 may act as a negative regulator of GPA1-mediated ABA responses in guard cells (Chen et al., 2006; Pandey and Assmann, 2004; Yadav et al., 2012). The roles of G-proteins signaling components in *Arabidopsis* abiotic stress response was validated recently where its functions were related to biotic and abiotic stress, hormone response and secondary metabolism; and consequently, the involvement of GCR1 as a key participant in those processes either independent of, or hitherto unattributed to G-protein was corroborated (Chakraborty et al., 2015b). This suggests that G-protein signaling pathway could offer novel common targets for the development of tolerance/resistance to multiple abiotic stresses allowing further genetic manipulation.

Overall, our findings complement previous studies in papers by Chakraborty et al. (2015b), Fredriksson and Schio (2005), Moriyama et al.(2006) and Taddese et al. (2014)and contribute to end the controversy of GPCRs in plant genomes. We expect that the increase of knowledge in plant genome allow the application and experimental validation of those candidates to contribute to the development of drought stress tolerance crops.

Conclusion

The strategy we described here overcomes the controversy about plant GPCR presence and strongly suggests the functionality of GPCR candidates in several taxonomic orders of plants. Moreover, the bioinformatic pipeline used in this work logically selected protein set for further experimental validation of their functions. Further studies would show the veritable structure-function relationship for a complex protein such as GPCRs could be determined by a multidisciplinary approach using both biochemical, structural determination and physiological approach or even genome editing.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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