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PLAC8 GENE FAMILY AND THEIR ROLE IN PLANTS AND MAMMALS

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Abstract
Programmed cell death (PCD) is a genetically organized cellular suicide, which play a fundamental role in a wide variety of developmental and physiological processes. Among the genes related to PCD/apoptosis control in humans, the PLAC8 gene (Placenta Specifies 8) presents an important role. In plants, this family was not previously described. We adopted a high throughput comparative genomic approach to conduct a broad survey of fully sequenced Viridiplantae genomes, to identify the presence of homologous genes coding for PLAC8 gene family. A total of 420 genes were identified, being 344 classified as type I and 76 as type II. The type I genes were found in plants, algae, fungi and mammals. On the other hand, the type II genes were identified only in plants, being distributed in bryophytes, pteridophytes, gymnosperms and angiosperms. These results suggested that type II genes could be resulted from duplication that occurred exclusively in plants.

INTRODUCTION
Programmed cell death (PCD) is a genetically regulated process of cellular suicide and is well known to play a fundamental role in a wide variety of developmental and physiological functions in animals, plants, and fungi. This process removes any mutated, infected or damaged cells from surrounding healthy tissue.A rapid increased in our knowledge about the role and regulation of animal PCD, especially considering apoptosis, has been occurring in the last years. These findings have been providing progression in a several diseases and resistance of cancers to therapeutic agents (Ameisen, 2002; Strasser, 2011). In mammals, a recently investigated gene related to apoptotic mechanism is known as PLAC8 (PLACenta specific 8). PLAC8 expression plays a role in differentiation, proliferation, and apoptosis in myeloid cells (Li et al., 2006; Rogulski et al., 2005; Wu et al., 2010). This gene also provides a mechanistic link between primary oncogenic mutations and the induction of autophagy. PLAC8 has been also described as a cooperation response gene, representing “a critical node in gene networks underlying the malignant phenotype” (McMurray et al. 2008). In plants, the investigation of PCD mechanisms using *Arabidopsis thaliana* pointed PLAC8 genes as potentially important in this process. Recently, Coll et al. (2011) proposed a deathsome, compound by several genes related with PCD regulation and control, as At1g52200 and At4g23470 genes, which possesses a typical PLAC8 motif. PLAC8 motif containing-protein has been identified in many species. In plants there are families of proteins with structural similarities to PLAC8 (Guo et al., 2010; Libault and Stacey, 2010). In tomato, the PLAC8-related gene, fruit weight 2.2 gene (fw2.2) regulates fruit weight by controlling cell number and several fw2.2-like

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genes present in other plants play a similar role (Guo et al., 2010; Libault and Stacey, 2010). Song et al. (2011) showed that PLAC8 motif containing-protein belong to a large gene family, which is present in fungi, algae, plants and animals. However, different family names were proposed to the proteins containing the PLAC8 motif: Onzin (Sherwin et al. 2000) and Cornifelin (Michibata et al. 2004) in humans, and FW2.2 (Fruit Weight2.2) (Frary et al.2000); PCR (Plant Cadmium Resistance) (Song et al. 2004); MCA1 (Mid1-complementing activity) (Nakagawa et al. 2007) and CNR (Cell Number Regulator) (Gou et al. 2010) in plants. Up till now, there is no devised nomenclature for naming the PLAC8 motif containing-proteins. Therefore, an existence of a PLAC8 family remains unclearly. The systematic sequencing of new plant species in recent years has uncovered the existence of several novel genes. Thus, naming genes families could not be simply arbitrary, since the context of comparative functional genomics has been allow to clarify what constitutes a gene family. The recognition of different names related to PLAC8 motif containing-proteins in many species has led us to a suggested revised nomenclature, which is based on the existing nomenclature of the first ever characterized PLAC8 gene in human.

MATERIAL AND METHODS

To identify genes PLAC8, the At1g52200 and At4g23470 were selected as bait against the Phytozome (http://phytozome.jgi.doe.gov/pz/portal .html) and PLAZA database. Metazome (https://metazome.jgi.doe.gov/pz/portal.html) and Fungi (https: / / fungi. ensembl.org/index .html) databases were used to identify sequences containing PLAC8 motif in humans and in fungi, respectively. The presence of conserved domains in the putative PLAC8 sequences identified was verified using the MEME software (http://meme.sdsc.edu/meme/). To analyze the existence of transmembrane domain, TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) software was used.

RESULTS AND DISCUSSION

Conceptually, gene families have a common ancestor, arise by gene duplication, and may share similar functions. Operationally, gene families can be defined by analysis of the sequence similarity and domain composition. However, a simple similarity threshold may be tendentious if the threshold inappropriately splits a divergent superfamily, or inappropriately groups together separate gene families (Cannon et al. 2004). Using BLAST approaches, a total of 420 genes containing the PLAC8 motif were identified. Interestingly, our phylogenetic results showed that PLAC1 and PLAC9 human genes, whose names suggest that they are related to PLAC8, have no relationship between themselves. PLAC1 (Placenta-specific 1) is a recently described X-linked gene and is expressed throughout human pregnancy by the differentiated trophoblast (Fant, 2010). Galaviz-Hernandez et al. (2003) reported the analysis of PLAC9 gene, which is weakly expressed though highly enriched in placenta. Thus, although presenting a similar acronym, PLAC1 and PLAC9 not compose the PLAC8 family, since they not present the PLAC8 motif. We propose a consistent nomenclature for all genes containing PLAC8 motif, based on the existing nomenclature of the first characterized PLAC8 gene in human. We showed that the previously described Onzin and Cornifelin genes are PLAC8-like. Several of the genes identified as containing the PLAC8 domain has been previously named as member of independent families, playing functions related to plant growth and plant resistance to stress. *Pcr1*, for example, was described as important for Cd (II)

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resistance (Song et al. 2004). Additionally, Cong et al. (2002) demonstrated that fw2.2 acts as a negative regulator of cell division during the very early stages of fruit development following pollination. Nakagawa et al. (2007) report the molecular identification of an integral plasma membrane protein MCA1 (Mid1-complementing activity) that correlates Ca2_ influx with mechanical stimulation in *Arabidopsis thaliana*. Also, Guo et al. (2010) showed that CNR1 reduced overall plant size when ectopically overexpressed and that plant and organ size increased when its expression was co-suppressed or silenced. Altogether, these genes containing the PLAC8 motif and present a very similar protein structure, suggesting a phylogenetic relation. Considering all genes identified, we observed two different groups of genes, being 344 classified as type I and 76 as type II. Phylogenetic analysis showed that type I and type II genes comprise two independent groups. Exon-intron analyze reinforce this grouping. The type I genes were found in plants, algae, fungi and mammals. In general, the PLAC8 motif is conserved in the majority of the sequences. Specially, a previously described important domain typical of PLAC8 domain was found, being this consensus sequence CCXXXXCPC or CLXXXXCPC well conserved. Song et al. (2004) showed that the domain CCXXXXCPC are situated in the N-terminal region of the protein and are important to Cd (II) resistance. Deletion or mutations in CC and CPC residues to AA and AAA strongly reduce the capacity of *AtPcr1* to confer Cd (II) resistance (Song et al. 2004). The complete Cys-rich region (but not a single Cys) in the first putative transmembrane domain of *AtPcr1* is important for Cd (II) resistance. Interestingly, in humans, this same cysteine-rich region between 28 and 38 amino acids of Onzin region is critical to antiapoptotic activity and allows the interaction of Onzin with Akt and with Mdm2 (leading to an inability to upregulate p53) (Rogulski et al. 2005). On the other hand, the type II genes were identified just in plants, being distributed in bryophytes, pteridophytes, gymnosperms and angiosperms. These results suggested that type II genes could be resulted from duplication that occurred exclusively in plants. To our knowledge, our study was the first to identify these genes, which represents an important novelty in plants. The PLAC8 motif was highly conserved in all sequences. However, these 76 type II genes presenta slight difference in the consensus sequence of PLAC8 domain. Instead the CCXXXXCPC or CLXXXXCPC consensus sequence, the CCXXXXCGP or CLXXXXCAP was found and is well conserved. If these sequences play some biological functions, similar to the other sequences previously showed, remains unclear. The analysis PLAC8 protein sequence showed that the majority of them present transmembrane domains. Cong and Tanksley (2006) localized the FW2.2 protein to the plasma membrane. Libaut et al. (2010) showed the presence of one putative transmembrane domain in soybean FWL1, suggesting that FWL1 might be targeted to the membrane. Song et al. (2004) showed that AtPcr1 has two hydrophobic segments, AA28-48 and AA51-71, which form helix structures that may acts as transmembrane domains. Also, MCA2 has at least two potential transmembrane segments (Nakagawa et al., 2007). Thus, our results are consistent with a structural feature previously described to PLAC8 motif containing-protein.

CONSCLUSION

In the present study, we described for the first time the PLAC8 gene family, which includes genes from the previously described Onzin, Cornifelin, FW2.2, PCR, MCA1, and CNR gene families. A consistent nomenclature for the genes containing PLAC8 domain was provided. We found that

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PLAC8 family is characterized by the presence of two groups of genes, type I and type II, which present prominent difference in the PLAC8 domain. We found that type I PLAC8 genes are widely distributed in plants, algae and fungi, while the type II PLAC8 genes are exclusively of plants. To our knowledge, the type II PLAC8 genes represent an important novelty in plants, since they were not described yet. Further investigations could be useful in order to describe the role of type II PLAC8 genes in plants.

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