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Genome-wide bioinformatics analysis of DELLA-family proteins from plants

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Abstract

DELLA-family proteins have been implicated as negative modulators of the gibberellin signaling pathway, which regulates many aspects of plant growth and development. Despite the importance of DELLA proteins, a genome-wide overview of the DELLA gene family is not yet available. Here, based on conserved domain searching, we identified 60 different DELLA-encoding genes from 29 plant genomes, including 49 complete length sequences. Phylogenetic analysis indicated that these DELLAs can be grouped into four different subfamilies, including Algae, Bryophyte, monocots and dicots. Analysis of domains and motifs in the DELLA gene family showed the following domains including DELLA, TVHYNP, VHIID, RKVATYFGEALARR, AVNSVFELH, RVER, and SAW are strictly conserved in the DELLAs. Gene duplication events were the main reason for expansion of the DELLA family; selective pressure operated on the DELLAs after gene duplication, resulting in the formation of distinct DELLA groups. Our results provide new insights into the evolutionary relationships of DELLA proteins.

Keywords: DELLA proteins; phylogeny; bioinformatic analysis; positive selection; plant.
Abbreviations: GA, Gibberellin; GAI, gibberellic acid insensitive; GID1, GIBBERELLIN INSENSITIVE DWARF1; GRAS, GAI, RGA, SCR; NCBI, National Center for Biotechnology Information; Ser/Thr motif, Serine/threonine motif; SH2, Src-homology 2.

Introduction

Gibberellins (GAs) are cyclic diterpenoid molecules that are involved in various essential development and growth processes in plants, including seed germination, hypocotyl elongation, shoot growth, leaf expansion, flowering initiation and flower organ development (Olszewski et al., 2002; Fleet and Sun, 2005; Yamaguchi, 2008). GAs were also shown to be involved in reproductive maturation (Evans and Poethig, 1995) and in survival in adverse environments (Achard et al., 2006). GAs can also enhance submergence-tolerance in some plants (Fukao and Bailey-Serres, 2008) and can dramatically affect plant architecture and biomass by integration with multiple hormone signaling networks (Patel and Franklin, 2009). The DELLA proteins, named after their conserved N-terminal D-E-L-L-A amino acid sequence, were originally identified as negative regulators of GA-induced growth, and they are a subset of the GRAS transcription factor family (Bolle, 2004). It has become clear that DELLAs do not bind directly to DNA as co-repressors or co-activators, but rather interact with other transcription factors to regulate GA-responsive gene expression (Lee et al., 2002; Achard et al., 2004; Cao et al., 2005; Achard et al., 2006; Achard et al., 2007). The degradation of DELLA proteins induced by GA in collaboration with the GA receptor GID1, and F-box protein mediates a key event in GA signaling (Fu et al., 2002; Feng et al., 2008). When GA is absent, DELLA proteins repress various GA responses in plants; when GA is present, GID1 binds to GA and triggers an interaction between GID1 and the DELLA protein. With the aid of the SCF complex, DELLA protein is then degraded via the 26S proteasome pathway, leading to various GA-dependent responses. This major pathway for GA-induced degradation of DELLA proteins has been reported in rice (Itoh et al., 2003) and barley (Gubler et al., 2002). It has also been reported that GA-induced degradation of DELLA proteins in *Arabidopsis* relieves a growth restraint (Dill et al., 2001; Silverstone et al., 2001b). This GA-DELLA mechanism regulates growth and development throughout the angiosperm life cycle (Richards et al., 2001). DELLAs are not only key components in a signal-transduction chain that regulates plant growth in response to GAs (Dill and Sun, 2001; Silverstone et al., 2001a; Alvey and Harberd, 2005) but also integrators of signals from additional growth-regulatory inputs. By altering the relative balance of salicylic acid and jasmonic acid signaling, DELLAs can also promote susceptibility to virulent biotrophs and resistance to necrotrophs (Navarro et al., 2008). DELLAs increase the activity of antioxidant enzymes and reduce production of reactive oxygen species that are involved in growth, signaling and pathogen responses, thereby indirectly mediating growth (Achard et al., 2008). Recent research has also revealed the role of DELLA repressors in several other novel response pathways, and DELLA proteins have been shown to function as repressors of the phytochrome interacting factor 3 (PIF3) and PIF4 transcriptional activators in the context of light-regulated seedling development (Schwechheimer and Willige, 2009). Ser/Thr motifs (poly S/T), Leu heptad repeats, putative nuclear localization signals, and a putative SH2 phosphotyrosine binding domain are the main characteristics of DELLA proteins (Gao et al., 2008). Ser/Thr motifs are possible sites for phosphorylation or glycosylation,

and Leu heptad repeats can mediate protein-protein interactions. It has been reported that the degradation of DELLA proteins in *Arabidopsis* first requires dephosphorylation, and the leucine zipper domain of the *Arabidopsis* DELLA proteins is essential for both their stability and activity (Wang et al., 2009). Deletion of the N-terminal DELLA motif in *Arabidopsis* DELLAs converts it into a GA-unresponsive, constitutively active repressor of GA signaling (Dill et al., 2001). The availability of plant genome sequences allows a timely and systematic genome-wide comparative and evolutionary analysis of gene families. The role of GRAS proteins in signal transduction and development in plants and the GA-DELLA growth-regulatory mechanism have been studied (Bolle, 2004). Detailed features, such as evolutionary relationships, gene structures, and protein motifs, for the DELLA family remain poorly understood, however, and these relationships are critical for understanding the characteristics of DELLAs. Here, we examined the evolution of the DELLA gene family from plants and conducted phylogenetic analyses to divide them into four subfamilies, followed by analysis of protein domains and motifs. We traced gene duplication events that most likely contributed to the expansion of the DELLA family. Positive selection analyses showed that some sites were under positive selection.

Results

Identification of DELLA genes in plants

The five known DELLA proteins in *A. thaliana* were used to perform multiple searches using blastp, keyword search, and domain search to identify all DELLA protein sequences in other plants. Subsequently, SMART and Pfam were used to filter the results to ensure that all genes identified as coding for DELLA proteins (Yasumura et al., 2007). Following this procedure, we were able to identify a total of 60 putative DELLA protein-coding genes in 29 different plants, including the five previously identified DELLA genes in *A. thaliana* (Table 1). Among them, 11 putative DELLA protein-coding genes showing shorter sequence length, indicate that they were part of complete protein sequences.

Phylogenetic and sequence characteristics of the DELLA protein family in plants

To better understand the phylogenetic relationship within the DELLA protein family, we constructed an ML phylogenetic tree (Fig. 1) based on the amino acid sequences of the conserved domains of the 49 DELLA protein-coding genes with complete open reading frame. For statistical reliability, we conducted bootstrap analysis with 1,000 replicates. This showed that bootstrap values were high in most of the nodes, thereby permitting subfamilies of DELLAs to be identified. Together with the topological structure of the phylogenetic tree, we classified the DELLA genes into four categories: I, II, III and IV. Of all the DELLAs we examined, the monocots (subgroup I) and dicots (II, III and IV) form distinct clades. This is strongly supported by their high bootstrap values and indicates that the gene expansion and extensive proliferation of DELLAs occurred after the monocot-dicot split. Moreover, among the three dicot subgroups, each DELLA group possesses at least one DELLA protein from each of Cruciferae, Leguminosae, Caricaceae, Euphorbiaceae and Rosebush, whereas Salicaceae and Vitaceae are only observed in subgroups II and III. This suggests that the most recent common ancestor of these dicot plants underwent diversification before the emergence of Cruciferae, Leguminosae, Caricaceae, Euphorbiaceae and rosebush. After the dicot/monocot split, dicot DELLAs underwent extensive diversification, and the main characteristics of the DELLA gene family in dicots probably had been established before the divergence of Salicaceae and Vitaceae from other dicots. Although our preliminary analysis of the known DELLA genes supports this possibility, at present the number of complete genome sequences for plants is insufficient to adequately measure the evolutionary history of the DELLA genes. The subsequent evolutionary patterns of the DELLA gene family in plants can be attributed to species-specific expansions. For dicots, most homologs belonging to each species (*e.g.* PpDELLAa, PpDELLAb in *Physcomitrella patens*) cluster together in their own subgroups (Fig. 1); for monocots, similar evidence was also found (*e.g.*, PtDELLA1, PtDELLA2), providing strong support for the occurrence of species-specific expansions. In such expansions, gene duplication following the differentiation of modern plants can be perceived as providing the main evolutionary patterns in the DELLA family, because genes at the terminal branches on the phylogenetic tree may represent recently duplicated genes (Xiong et al., 2005). Given the limited number of DELLAs identified in these plants, however, it is difficult to determine whether segmental or tandem duplication contributed to the evolution of DELLAs.

Analysis of domains and motifs in the DELLA gene family

To better understand the function and phylogenetic relationship with motif composition, we performed throughput domain and motif analysis for all protein sequences with complete length within the DELLA gene family. The software hmmpfam, part of the HMMer package (Eddy, 1998), was used to initially perform domain searches in all of the identified DELLAs (Supplementary Table S1). As expected, most identified DELLA family members contain the common GRAS domain—a transcription factor domain involved in development and other processes (Pysh LD, Wysocka-Diller JW, 1999) - which suggests a major functional role for DELLAs in the GA signaling pathway. Analysis of domain composition cannot be used to reveal detailed and dispersed sequence patterns in proteins, and thus it was not possible to accurately define a motif in DELLAs only using the domain search tools in hmmpfam. We therefore performed an MEME motif search for each of the DELLA sequence subfamilies separately. The motifs found by MEME were compared with the domain searched by SMART and Pfam. Using this method, we identified some important motifs in DELLAs (Fig. 2 and Supplementary Table S2). Despite a few differences of some amino acids, all other motifs (DELLA, TVHYNP, VHIID, RKVATYFAEALARR, RVER, and SAW) are strictly conserved in the DELLAs (Supplementary Table S2), implying their import role in biological function. For example, both the DELLA and TVHYNP motif are necessary for restriction to GA-promoted processes (Itoh et al., 2002), the VHIID motif is the putative DNA-binding domain that plays an important role in GA and abscisic acid pathways (Bassel et al., 2008), and deletion of the N-terminal 108 residues (encompassing the DELLA and TVHYNP motifs) slows RGA degradation (Wang et al., 2009). The four known motifs, namely 1 (VHIID), 2 (RVER), 4 (RKVATYFAEALARR), and 6 (SAW) (Fig. 2), as well as other previously unknown motifs (3, 5, 9, 10, 11, 12, 13, 14, 15) are highly conserved at the C-terminal end of all DELLAs, indicating that this region may have a fundamental function in all DELLAs (Dill et al., 2004). On the other hand, all the DELLA subfamilies contain the two signature motifs

Table 1. A list of DELLA-family proteins from plants.

Name	Accession no NCBI Nr database	Species		Name	Accession no.	Species
AtGAI	CAA75492	*Arabidopsis thaliana*		BoGRAS	BAG16374	*Brassica oleracea*
AtRGA	CAA72177	*Arabidopsis thaliana*		BrRGA1	Q5BN23	*Brassica rapa*
AtRGL1	NP_176809	*Arabidopsis thaliana*		BrRGA2	Q5BN22	*Brassica rapa*
AtRGL2	NP_186995	*Arabidopsis thaliana*		CmGAIP	Q6EI06	*Cucurbita maxima*
AtRGL3	NP_197251	*Arabidopsis thaliana*		CmGAIP-B	Q6EI05	*Cucurbita maxima*
DaGAI-A	AAM15898	*Dubautia arborea*		GmDELLA1	XM_00353834	*Glycine max*
DaGAI-B	AAM15880	*Dubautia arborea*		GmDELLA2	XM_003552932	*Glycine max*
GbGAI	ABG26370	*Gossypium barbadense*		GmDELLA3	NM_001254019	*Glycine max*
GhGAI	Q84TQ7	*Gossypium hirsutum*		GmDELLA4	XM_003528233	*Glycine max*
GhRGA	AAY28970	*Gossypium hirsutum*		GmDELLA5	XM_003531105	*Glycine max*
HvSLN1	Q8W127	*Hordeum vulgare*		GmDELLA6	XM_003523953	*Glycine max*
LeGAI	Q7Y1B6	*Lycopersicon esculentum*		GmDELLA7	XM_003535403	*Glycine max*
LsDELLA1	BAG71200	*Lactuca sativa*		GmGAI1	ABO61516	*Glycine max*
LsDELLA2	BAG71201	*Lactuca sativa*		MdDELLA1	AAY56752	*Malus x domestica*
MhDELLA	ABS50250	*Malus hupehensis*		MdDELLA2	AAY56751	*Malus x domestica*
MhGAI1	ABL61270	*Malus hupehensis*		MdDELLA3	AAY56750	*Malus x domestica*
OsSLR1	NP_001051032	*Oryza sativa*		MdDELLA4	AAY56749	*Malus x domestica*
PpDELLAa	XM_001774262	*Physcomitrella patens*		MdDELLA5	AAY56754	*Malus x domestica*
PpDELLAb	XM_001754038	*Physcomitrella patens*		MdDELLA6	AAY56753	*Malus x domestica*
PsCRY	ABI34432	*Pisum sativum*		PvDELLA1	BAF62636	*Phaseolus vulgaris*
PsDELLA	ABI30654	*Pisum sativum*		PvDELLA2	BAF62637	*Phaseolus vulgaris*
PtDELLA1	XM_002312414	*Populus trichocarpa*		RcGAI	XP_002534030	*Ricinus communis*
PtDELLA2	XM_002314763	*Populus trichocarpa*		RcGAI1	XP_002529354	*Ricinus communis*
PtDELLA3	XM_002305162	*Populus trichocarpa*		RcGAIP-B	XP_002527794	*Ricinus communis*
PtDELLA4	XM_002302939	*Populus trichocarpa*		SbDella	XP_002466594	*Sorghum bicolor*
SkDELLA	ABU63412	*Selaginella kraussiana*		VvDELLA1	XM_002266231	*Vitis vinifera*
SmDELLA	ABX10758	*Selaginella moellendorffii*		VvGAI1	XM_002284612	*Vitis vinifera*
SoGAI	AAZ08571	*Saccharum officinarum*		WgGAI-B	AAM15886	*Wilkesia gymnoxiphium*
SpDELLA	ABU63411	*Sphagnum palustre*		ZmD8	Q9ST48	*Zea mays*
TaRhtD1a	Q9ST59	*Triticum aestivum*		ZmD9	ABI84225	*Zea mays*

Table footnote: At, *Arabidopsis thaliana*; Bo, *Brassica oleracea*; Br, *Brassica rapa*; Cm, *Cucurbita maxima*; Da, *Dubautia arborea*; Gb, *Gossypium barbadense*; Gh, *Gossypium hirsutum*; Gm, *Glycine max*; Hv, *Hordeum vulgare*; Le, *Lycopersicon esculentum*; Ls, *Lactuca sativa*; Md, *Malus x domestica*; Mh, *Malus hupehensis*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Ps, *Pisum sativum*; Pt, *Populus trichocarpa*; Pv, *Phaseolus vulgaris*; Rc, *Ricinus communis*; Sb, *Sorghum bicolor*; Sk, *Selaginella kraussiana*; Sm, *Selaginella moellendorffii*; So, *Saccharum officinarum*; Sp, *Sphagnum palustre*; Vv, *Vitis vinifera*; Wg, *Wilkesia gymnoxiphium*; Ta, *Triticum aestivum*; Zm, *Zea mays*

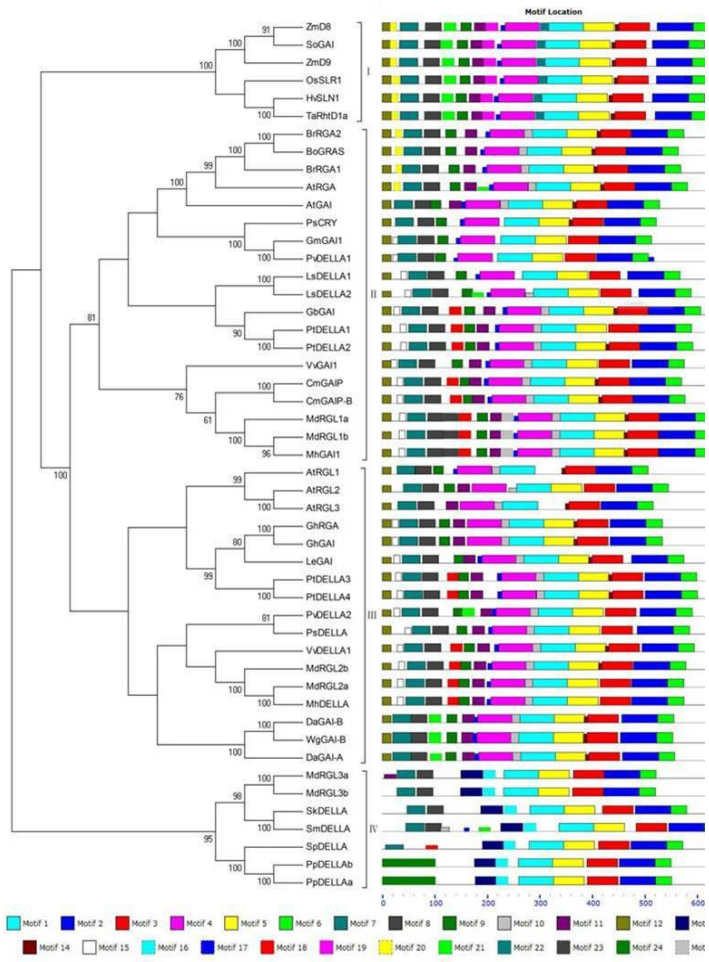

Fig 1. Phylogenetic tree and motif structure of the DELLA protein family in plants. The DELLA proteins were classified into four categories: I, II, III and IV, the monocots (subgroup I) and dicots (II, III and IV). In all motifs detected, DELLA, TVHYNP, VHIID, RKVATYFAEALARR, RVER, and SAW are strictly conserved in the DELLAs.

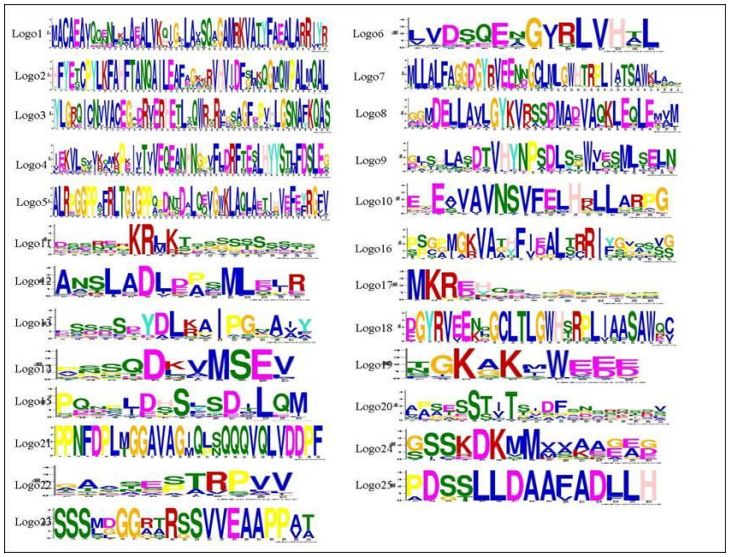

Fig 2. All motif sequences identified in the DELLA proteins. The overall height of each stack indicates the sequence conservation at that position, whereas the height of symbols within each stack reflects the relative frequency of the corresponding amino acid.

DELLA and TVHYNP—except for PpDELLAa and PpDELLAb, which do not contain an obvious DELLA domain. In *P. patens*, the DELLA motif appears to have been mutated or lost, a possible explanation is that the DELLAs may function differently in these plants.

Positive selection inferred by analysis of DELLA sequences

To determine whether the DELLA gene family had experienced positive selection pressure in its evolutionary history, we performed positive selection analysis on the DELLA genes. First, the phylogenetic tree based on nucleotide sequences was constructed with DELLA gene coding sequences using the neighbor-joining method implemented in MEGA 4.0 (Fig. 3). Partial sequences were excluded to avoid possible analysis biases. To test for variable ω ratios among lineages, the LRT was conducted to compare the two extreme models: a one-ratio model that assumes a fixed rate ratio for all branches, and a free-ratio model that assumes an independent ω ratio for each branch (Yang, 1997; Yang et al., 2008). The resulting logarithm of the likelihood value for a one-ratio model is –10714.576446, and for a free-ratio model is –9987.939992, with twice the log likelihood difference (2Δl) equal to 1453.272908. It is clear that some branches of the DELLA phylogenetic tree have $\omega > 1$, which shows evidence for adaptive evolution. If a new gene family member is produced by duplication events, it would either evolve a new function driven by positive selection or be lost during evolution. In contrast to a previous report that implied that significant heterogeneity in the evolutionary rate of GA pathway genes is mainly ascribed to differential constraint relaxation rather than positive selection (Yang et al., 2009), our study shows that positive selection acted on the DELLA gene family during its evolutionary history.

Discussion

Phylogenetic analysis revealed that the DELLA proteins were classified distictly into different groups, including angiosperm, gymnosperms, lycophyte, and bryophyte. This showed that DELLA genes may experienced the evolutionary history from lower plants to higher plants (Yasumura et al. 2007). Domain and motif analysis showed that lower plants including lycophyte, and bryophyte lack conserved regions, such as SkDELLA contains divergent but conserved domains, PpDELLAa and PpDELLAb are more widely divergent in several conserved domains (Figure 1 and 3; Tables S1 and S2). These results were consistent with the researches that exogenous GA3 (which promotes angiosperm and gymnosperm growth) did not detectably promote the growth of *S. kraussiana* (sporophyte) or *P. patens* (gametophyte) (Pharis and Owens 1966; Yasumura et al. 2007). And DELLA-deficient *P. patens* mutant strain lacks the derepressed growth characteristic of DELLA-deficient angiosperms, and that both *S. kraussiana* and *P. patens* lack detectable growth responses to GA (Yasumura et al. 2007). Conserved DELLA domain is one of key components that function in promoting growth and development. Mutant of DELLA protein in wheat and arabidopsis results a dwarf phenotype (Peng et al. 1999; Cao et al. 2005; Achard et al. 2007). Of course, the exact functions of DELLA proteins from lower plants to higher plants still need further experiments confirmation.

Materials and methods

Sequence data

The *A. thaliana* DELLA protein sequences (Accession No:

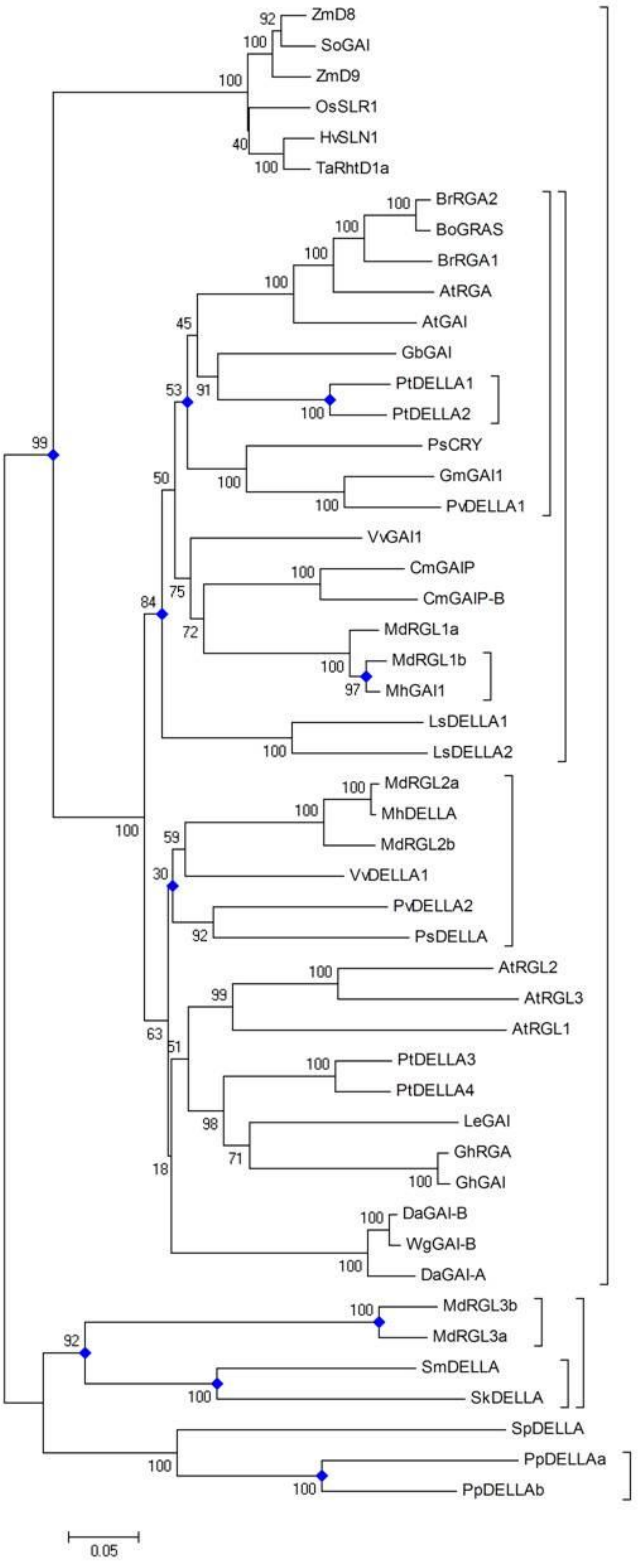

Fig 3. Phylogenetic tree reconstructed from plant DELLA nucleotide sequences using the neighbor-joining method. The number next to the branches represents bootstrap values ≥ 50% based on 1,000 resamplings. The scale bar shows total nucleotide distance. Blue dots indicate branches with rates of numbers of nonsynonymous and synonymous substitutions >1.

CAA75492, CAA72177, NP_176809, NP_186995, NP_197251) reported by Peng et al. (1997), Silverstone et al. (1998), Wen and Chang (2002) were retrieved from The *Arabidopsis* Information Resource (http://www.arabidopsis.org/). To identify members of the DELLA protein family in other plants, five DELLA sequences from *Arabidopsis thaliana* and one DELLA protein sequences from wheat as queries were used as perform domain searches, as well as multiple database searches, with the blastp programs available on NCBI nonredutant protein sequences databases [Organism: plants (taxid:3193)] (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The SMART and Pfam tools were employed to detect the conserved domains with default parameters, respectively. DELLA gene sequences corresponding to above-queried protein sequences were downloaded from NCBI gene sequence databases.

Multiple sequence alignment and phylogenetic tree reconstruction

Multiple alignments of amino acid sequences were generated using ClustalX v1.83, Sequence relationships were inferred using the Maximum likelihood (ML) method with default parameters (Guindon and Gascuel, 2003). Using PhyML v2.4, ML phylogenies were reconstructed. The bootstrap value inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed (Sanderson and Wojciechowski, 2000). Branches corresponding to partitions reproduced in fewer than 60% of the bootstrap replicates were collapsed. Assignment of the DELLAs to the different subfamilies was performed on the basis of their similarity and grouping in phylogenetic trees.

Identification of motifs in DELLAs

To identify motifs shared among related proteins within the DELLA protein family, we used the MEME motif search tool (Bailey and Elkan, 1995) with the default settings, except the maximum number of motifs to be found was set at 25, and the maximum width was set at 100. The resulting motifs found by MEME were then annotated using SMART and Pfam.

Positive selection analysis of DELLA sequences

Adaptive evolution analyses were performed using the Codeml program implemented in the PAML v4.0 software package (Yang, 1997). For the DELLA genes, the protein-coding sequences were aligned based on the translated protein sequences using the ClustalW program in MEGA 4.0 (Tamura et al., 2007). PAML was then used to detect selective pressure among these sequences using the branch models (Yang, 2007). The one ratio model (M0) assumes a single ratio model for all branches and all sites, whereas the free ratio model (Mf) assumes an independent ratio for each branch of the tree. A likelihood ratio test (LRT) was then conducted to determine whether there was statistically significant heterogeneity between the two models and whether the ω ratios were different. If the LRT is significant, the null hypothesis that two models are not significantly different is rejected, and the model with the higher LRT is assumed to be a better model (Yang and Nielsen, 1998; Bielawski and Yang, 2003). The LRT was also used to determine whether the ω ratios were different within lineages of the two models.

Conclusion

Our results revealed phylogenetic relation of DELLA-proteins from plants in genome scale. Phylogenetic analysis indicated that these DELLAs can be grouped into four different subfamilies, including Algae, Bryophyte, monocots and dicots. Gene duplication events were the main reason for expansion of the DELLA family; selective pressure operated on the DELLAs after gene duplication, resulting in the formation of distinct DELLA groups. Analysis of domains and motifs found that DELLA, TVHYNP, VHIID, RKVATYFGEALARR, AVNSVFELH, RVER, and SAW are strictly conserved in the DELLAs.

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