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Full Length Article

Genome-wide Identification and Analyses of the Rice OsDUF639 Family

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Abstract

With the advance of sequencing technology, the number of sequenced plant genomes has been rapidly increasing. However, functional study of the genes in these sequenced genomes lags far behind; as a result, many plant proteins in public databases are recognized as proteins with domains of unknown function (DUF). DUF639 is such a protein family that consists of plant proteins with unknown function. In this study, we attempted to uncover the biological functions of eight DUF639 members (OsDUF639.1-OsDUF639.8) in rice Nipponbare. Specifically, using phylogenetic analysis, we classified these proteins into four groups (group I, II, III and IV). Next, to find out whether these proteins have tissue-specific expression preference, we examined the expression of all these proteins in 15 distinct rice tissues. We found that OsDUF639.3 was preferentially expressed in grain tissues. The expression of these eight proteins under various abiotic stresses was also examined. The results indicated that the expression of OsDUF639.6 remained unchanged approximately under various abiotic stress conditions, while under heat conditions, the expression of OsDUF639.1, OsDUF639.2, OsDUF639.3, OsDUF639.4 and OsDUF639.7 was significantly elevated. Furthermore, we found that overexpression of OsDUF639.1 and OsDUF639.4 in *E. coli* may significantly improve resistance to heat, whereas overexpression of OsDUF639.2, OsDUF639.3 and OsDUF639.7 do not have a similar effect. In conclusion, our results suggest that DUF639 proteins may have different tissue-specific expression and diversified functions in rice, with some of them possibly contributing to heat resistance.

Keywords: Rice; DUF; Gene family; Expression; Abiotic stress; Overexpression

Introduction

A number of abiotic stresses conditions, including heat, drought and salt, can exert strong negative effects on the productivity of crops. To counteract these negative effects, plants have developed a lot of strategies to adapt to adverse environments (Ingram and Bartels, 1996; Zhu, 2002).

Heat stress is one of the restricting factors that can cause severe crop-yield losses, especially in recent years (Yokotani *et al.*, 2008). Heat stress usually affects plant growth, development, photosynthesis and respiration and is often accompanied by the generation of reactive oxygen species (ROS) (Mittler, 2002; Song *et al.*, 2014; Ohama *et al.*, 2016). At present, people's research on the heat stress response is mainly focused on the heat shock proteins (HSP) and reactive oxygen species (ROS)-scavenging enzymes in plants. Although heat stress induces oxidative stress, plants have developed a series of enzymatic systems, including ROS-scavenging enzymes, to counteract ROS (Scandalios, 1993; Vanderauwera *et al*., 2011; Ohama *et al.*, 2016).

As heat stress has been causing severe adverse effects on plant growth in recent years, it is urgent to reveal the molecular mechanism of plant heat stress response. At present, some genes have been used to improve the heat tolerance in rice (Fang *et al.*, 2015; Shiraya *et al.*, 2015; Liu *et al.*, 2016), but the number of such genes still remain rather small.

The domain of unknown function (DUF) is a kind of protein domain with unknown function (Bateman *et al.*, 2010; Finn *et al.*, 2016). In recent years, some DUF-containing proteins were reported to enhance the tolerance to drought and salt stress in plant (He *et al.*, 2011; Luo *et al.*, 2014; Wang *et al.*, 2014; Guo *et al.*, 2016). DUF639 family members have an average length of around 210 residues. To our knowledge, none of the members in this protein family have been functionally characterized in rice.

In the present study, the protein sequences of the OsDUF639 family in rice were analyzed and the expression profile of *OsDUF639* family members was also examined. Furthermore, the genes of five OsDUF639 proteins (*OsDUF639.1*, *OsDUF639.2*, *OsDUF639.3*, *OsDUF639.4* and *OsDUF639.7*) were overexpressed in *Escherichia coli*.

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Materials and Methods

Database Searches and Sequence Analysis

We obtained all the gene sequences encoding OsDUF639 family proteins from the rice genome annotation project database (http://rice.plantbiology.msu.edu/) using domain number PF04842. Information for each *OsDUF639* family member was obtained from RGAP. The protein localizations of OsDUF639 members were predicted using online protein location prediction software TargetP 1.1 (Emanuelsson *et al.*, 2007). Protein conservative structural analysis software MEME version 4.9.1 was used to analyze the motifs of OsDUF639 family members (Bailey *et al.*, 2009). MEGA4 was used to study the relationships among the DUF639 family members in evolution (Tamura *et al.*, 2007).

Various Stress and ABA Treatment

After rice Nipponbare seeds were germinated, the resulted seedlings were grown hydroponically in a 30 dm^3 nutrient solution vessel as described previously (Li *et al.*, 2010). Then the four-leaf stage seedlings of rice Nipponbare were are exposed in various stress conditions as described previously (Li *et al.*, 2017).

Real Time PCR

Total RNA of rice seedlings was extracted according to the manufacturer's instructions with Trizol reagent (GIBCO/BRL). Real-time PCR was performed as described before (He *et al.*, 2017). We used the rice *Actin1* gene (*LOC_Os03g50885*) as an internal control. Data analyses of the relative expression levels was performed using the $2^{-\Delta\Delta Ct}$ method as described previously (Livak and Schmittgen, 2001). Then we confirmed all the quantitative PCR products by sequencing.

Assay for Heat Tolerance of *E. coli* Transformants

The transformed *E. coli* Rosetta cells were grown at 37°C overnight in Luria-Bertani (LB) liquid medium containing 100 μg/cm^3 of ampicillin (LaVallie *et al.*, 1993). Isopropylthio-β-D-galactoside (IPTG) was used to induce the expression of the transformed gene.

In the heat tolerance test, at 0, 0.5, 1, 1.5, 2, 2.5 h after heat stress treatment (50°C water bath) respectively, 100 μL of dilutions (1:100) was spotted onto LB agar plates with 1 mM IPTG.

Data Analysis

The experiment was arranged as a completely randomized design and all the experiments were repeated three times. Data were statistically analyzed by *ANOVA* and the data from the different treatments were compared using Duncan's Multiple Range Test at 5% probability.

Results

Sequence Analysis of OsDUF639 Family Members

We obtained eight genes (*OsDUF639.1* to *OsDUF639.8*) encoding OsDUF639 family proteins (Table 1). They are distributed on chromosome 1 (*OsDUF639.1*), 2 (*OsDUF639.2*), 3 (*OsDUF639.3*), 4 (*OsDUF639.4* and *OsDUF639.5*), 5 (*OsDUF639.6*), 6 (*OsDUF639.7*), and 10 (*OsDUF639.8*), respectively.

Information about OsDUF639 family members is presented in Table 1. The length of OsDUF639 proteins varies from 312 (OsDUF639.4 and OsDUF639.7) to 831 (OsDUF639.1) amino acids. In order to identify sequences conserved among the eight OsDUF639 members, the MEME motif search tool was applied and we found three distinct motifs (Fig. 1A). Motif 1, motif 2 and motif 3 were found in OsDUF639.1, OsDUF639.2, OsDUF639.3, OsDUF639.5, OsDUF639.6 and OsDUF639.8. Interestingly, in OsDUF639.4 and OsDUF639.7, motif 2 was not present (Fig. 1B).

Phylogenetic Analysis of OsDUF639 Family

Here five *Arabidopsis* DUF639 members, AT1G48840.1 (located in cytosol), AT1G71240.1 (located in mitochondrion), AT2G21720.1 (located in plasma membrane), AT3G18350.1 (located in cytosol) and AT5G23390.1 (located in peroxisome), were considered as reference sequences. The results showed that the OsDUF639 family members can be divided into four major groups (I, II, III and IV) (Fig. 2). Group I is composed of five members (OsDUF639.2, OsDUF639.4, OsDUF639.5, OsDUF639.7, and AT5G23390.1), group II consists of four members (OsDUF639.6, OsDUF639.8, AT1G48840.1 and AT3G18350.1), group III includes two members (OsDUF639.1 and AT1G71240.1) and group IV comprises two members (AT2G21720.1 and OsDUF639.3).

Expression Profile of *OsDUF639* Members in Different Tissues and Various Stress Conditions

In this study, we examined the expression of the eight *OsDUF639* protein-encoding genes in 15 rice tissues. Under normal conditions, the eight genes showed different expression profile (Fig. 3). Particularly, gene *OsDUF639.3* was preferentially expressed in Em1 (embryo at 7 days after flowering), Em2 (embryo at 28 days after flowering) and En2 (endosperm at 28 days after flowering). The expression profiles of *OsDUF639.1*, *OsDUF639.4* and *OsDUF639.7* are quite similar: the highest expression in Lb2 (leaf blade from plants with four tillers), while the lowest in En2 (endosperm at 28 days after flowering).

Table 1: *OsDUF639* gene family and their predicted protein structure information

name	MSU locus	FL-cDNA accession no.	AA length	introns	location
OsDUF639.1	LOC_Os01g64870	AK121602	831	10	Mitochondrion
OsDUF639.2	LOC_Os02g42530	AK072512	735	9	Mitochondrion
OsDUF639.3	LOC_Os03g06180	AK106245	685	9	Any other location
OsDUF639.4	LOC_Os04g55780	not found	312	2	Any other location
OsDUF639.5	LOC_Os04g58100	AK069752	718	9	Any other location
OsDUF639.6	LOC_Os05g17990	not found	696	8	Any other location
OsDUF639.7	LOC_Os06g45930	not found	312	2	Any other location
OsDUF639.8	LOC_Os10g03830	AK072882	698	8	Any other location

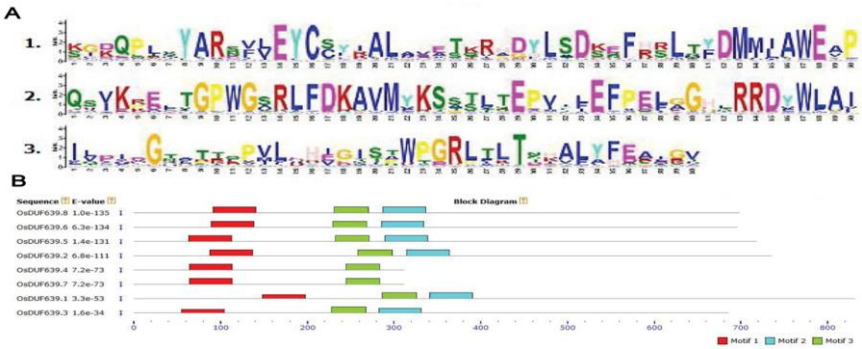

Fig. 1: Conservative structural analysis of rice OsDUF639 family. Moif 1, motif 2, and motif 3 were conserved motifs in rice OsDUF639 family obtained by MEME (A). Distribution of conserved motifs in OsDUF639 proteins identified by MEME software (B)

In this study, the expression profile of *OsDUF639* family under various abiotic stress conditions was also examined (Fig. 4). The expression of *OsDUF639.6* remained unchanged under various stress and ABA conditions. In contrast, under heat conditions, the expression of *OsDUF639.1*, *OsDUF639.2*, *OsDUF639.3*, *OsDUF639.4* and *OsDUF639.7* was significantly elevated, while the expression of *OsDUF639.5* displayed significant decrease. The expression of *OsDUF639.2* decreased significantly, whereas the expression of *OsDUF639.5* and *OsDUF639.8* rise dramatically under drought conditions. Under salt conditions, the expression of *OsDUF639.1* and *OsDUF639.8* was significantly elevated, while the expression level of *OsDUF639.2* and *OsDUF639.3* displayed significant decrease. Under cold conditions, the expression of *OsDUF639.5* went up significantly, while the expression level of *OsDUF639.4* and *OsDUF639.7* displayed significant decline. Under ABA treatment, only gene *OsDUF639.1* was upregulated, while the expression level of other members of *OsDUF639* family remained unchanged (Fig. 4).

OsDUF639.1 and *OsDUF639.4* Improved Heat Resistance in Transgenic *E. coli*

We hypothesized that genes *OsDUF639.1*, *OsDUF639.2*, *OsDUF639.3*, *OsDUF639.4* and *OsDUF639.7* may be involved in response to heat stress. So these genes were overexpressed in *E. coli*.

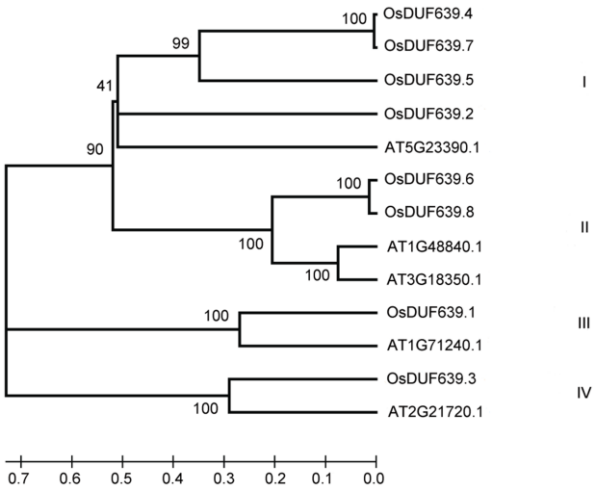

Fig. 2: Phylogenetic relationship of eight OsDUF639 members in rice and five OsDUF639 members in *Arabidopsis*. The unrooted tree was generated using MEGA4.0 program by Maximum-likelihood method

Under normal conditions, there was no evident difference in the number of colonies between transgenic lines (*E. coli* transformed with *pET32a-OsDUF639.1*, *pET32a-OsDUF639.2*, *pET32a-OsDUF639.3*, *pET32a-OsDUF639.4*, *pET32a-OsDUF639.7*), and the control (*E. coli* transformed with *pET-32a*). Under heat conditions, the number of clones of *E. coli* recombinants overexpressing

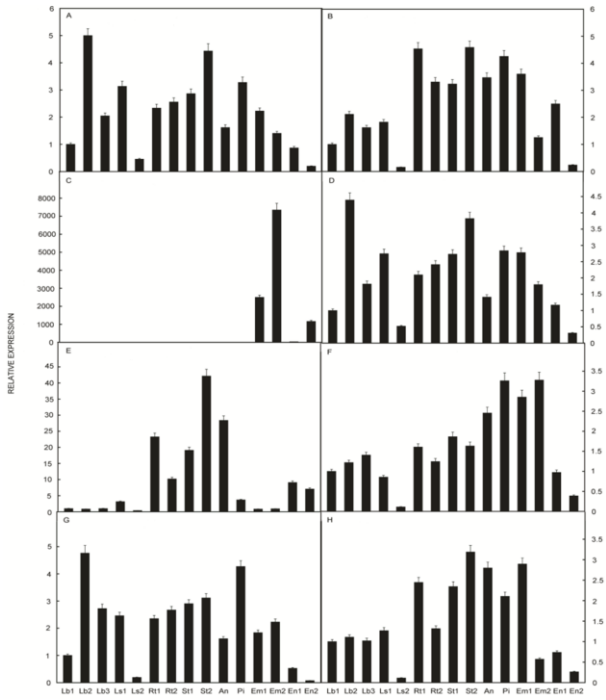

Fig. 3: Real time PCR analysis of *OsDUF639* gene family members (A-H represents gene *OsDUF639.1* to *OsDUF639.8*) in rice Nipponbare different tissues. Fifteen representative tissues are as follows: Lb1, leaf blade at four-leaf stage; Lb2, leaf blade from plants with four tillers; Lb3, leaf blade at ripening stage; Ls1, Leaf sheath at four-leaf stages; Ls2, Leaf sheath from plants with four tillers; Rt1, root at four-leaf stages; Rt2, root at from plants with four tillers; St1, stem from plants with four tillers; St2, stem at ripening stage; An, 1.2-1.5 mm anther; Pi, pistil from 10-14 cm inflorescence; Em1, embryo at 7 days after flowering; Em2, embryo at 28 days after flowering; En1, endosperm at 7 days after flowering; En2, endosperm at 28 days after flowering. The rice *Actin1* transcript levels were used as internal controls. Error bars indicate SE based on three biological replicates

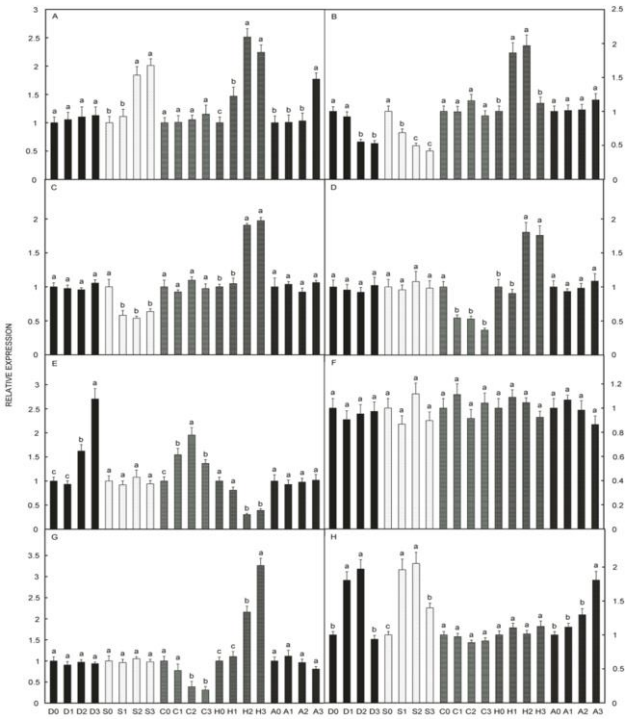

Fig. 4: Relative expression levels of *OsDUF639* gene family members (A-H represents gene *OsDUF639.1* to *OsDUF639.8*) in rice Nipponbare under various stress conditions and ABA treatment. For salt stress and drought stress, seedlings were sampled at 0, 4, 8, and 16 h, respectively. For ABA, heat and cold stress, seedlings were sampled at 0, 1, 3, 8 h, respectively. D, drought; S, salt; C; cold; H, heat; A, ABA. The rice *Actin1* transcript levels were used as internal controls. Error bars indicate SE based on three biological replicates. For each stress treatment, columns with different letters indicate significantly different values at $P < 0.05$

pET-32a, *pET32a-OsDUF639.2*, *pET32a-OsDUF639.3* and *pET32a-OsDUF639.7* was very close (Fig. 5B), while transgenic colonies transformed with *pET32a-OsDUF639.1* and *pET32a-OsDUF639.4* outnumbered the control (Rosetta/pET-32a) at 0.5, 1, 1.5, 2, 2.5 h after heat treatment respectively (Fig. 5A). These results indicated that the overexpression of *OsDUF639.1* and *OsDUF639.4* in *E. coli* may significantly improve resistance to heat, whereas the overexpression of *OsDUF639.2*, *OsDUF639.3* and *OsDUF639.7* do not have similar effect.

Discussion

OsDUF639 Family Members Play Different Roles

The expression of *OsDUF639* family in different tissues was examined and the results indicated that the expression patterns of the *OsDUF639* family members were vastly different (Fig. 3). Particularly, as *OsDUF639.3* was preferentially expressed in grain tissues, such as Em1, Em2 and En2, we speculated that this gene may be involved in rice grain development.

In general, gene family members display similar increase or decrease under various stresses conditions (Dubouzet *et al.*, 2003; Ito *et al.*, 2006; Su *et al.*, 2010). In this study, the expression of *OsDUF639* family under abiotic stresses conditions and ABA treatment was examined, and surprisingly, we found the eight members of *OsDUF639* family in rice displayed greatly distinct expression patterns under various stresses conditions and ABA treatment (Fig. 4). We thus suggest that *OsDUF639* family members may play different roles in response to various stresses.

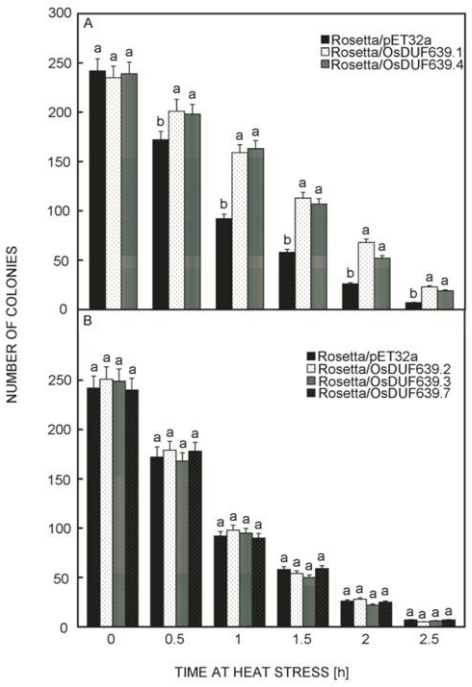

Fig. 5: Growth effect of *E. coli* recombinants overexpressing *OsDUF639.1*, *OsDUF639.4* (A) and *OsDUF639.2*, *OsDUF639.3*, and *OsDUF639.7* (B) under heat stress. The number of the control (Rosetta/pET-32a), Rosetta/*OsDUF639.1*, Rosetta/*OsDUF639.4*, Rosetta/*OsDUF639.2*, Rosetta/*OsDUF639.*3, Rosetta/*OsDUF639.7* colonies was counted. Error bars indicate SE based on three biological replicates. For each heat stress treatment time, columns with different letters indicate significantly different values at $P < 0.05$

OsDUF639. 1 and *OsDUF639.4* Improved the Tolerance to Heat in Transgenic *E. coli*

In this study, the growth effect of *E. coli* recombinants under heat stress was determined and the results indicated that overexpression of *OsDUF639.1* and *OsDUF639.4* in *E. coli* can significantly improve resistance to heat (Fig. 5). So far, the specific mechanism by which heat tolerance is enhanced in *E. coli* recombinants transformed with *OsDUF639.1* and *OsDUF639.4* remains unclear. The overexpression of some heat shock protein genes can enhance heat tolerance to *E. coli* (Yeh *et al.*, 1997; Liu *et al.*, 2009; Zhang *et al.*, 2016). Under heat conditions, it is possible that OsDUF639.1 and OsDUF639.4 interact with some heat shock protein, thus better starting the heat shock reaction in transgenic *E. coli*. Another possibility is that the heat stress signal was strengthened in *E. coli* recombinants transformed with *OsDUF639.1* and *OsDUF639.4*, and the enhanced heat stress signal promotes the increased expression of a lot of heat stress-related genes, leading to better improvement in heat tolerance in transgenic *E. coli*.

The functions of some DUF proteins have been elucidated recently. *TBL3* (*TRICHOME BIREFRINGENCE-like3*), encoding a protein with a DUF231 domain, contributes to the synthesis and deposition of secondary wall cellulose (Bischoff *et al.*, 2010). Some *DUF724* gene family members in Arabidopsis may be involved in the polar growth of plant cells via transportation of RNAs (Cao *et al.*, 2010). Several *DUF784* genes are involved in *Arabidopsis* embryo sac development (Jones-Rhoades *et al.*, 2007) and some *DUF1218* family members have been implicated in several aspects of cell wall biology (Persson *et al.*, 2005). In this study, *OsDUF639.1* and *OsDUF639.4* improved heat resistance in transgenic *E. coli*. DUF-containing proteins can play different roles in plants.

Conclusion

We provided important sequence information regarding the *OsDUF639* family members and the expression profile of *OsDUF639* family members. Our results suggest that *OsDUF639.1* and *OsDUF639.4* may improve the resistance to heat stresses in transgenic *E. coli.* All the results obtained in this study improve the understanding of the poorly-studied OsDUF639 proteins, and have potential to inform future studies on other proteins that contain DUFs.

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