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ORIGINAL PAPER

Functional relevance of J-protein family of rice (*Oryza sativa*)

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Abstract Protein folding and disaggregation are crucial processes for survival of cells under unfavorable conditions. A network of molecular chaperones supports these processes. Collaborative action of Hsp70 and Hsp100 proteins is an important component of this network. J-proteins/DnaJ members as co-chaperones assist Hsp70. As against 22 DnaJ sequences noted in yeast, rice genome contains 104 J-genes. Rice J-genes were systematically classified into type A (12 sequences), type B (9 sequences), and type C (83 sequences) classes and a scheme of nomenclature of these proteins is proposed. Transcript expression profiles revealed that J-proteins are possibly involved in basal cellular activities, developmental programs, and in stress. Ydj1 is the most abundant J-protein in yeast. Ydj1 deleted yeast cells are nonviable at 37 °C. Two rice ortholog proteins of yeast Ydj1 protein namely OsDjA4 and OsDjA5 successfully rescued the growth defect in mutant yeast. As Hsp70 and J-proteins work in conjunction, it emerges that rice J-proteins can partner with yeast Hsp70 proteins in functioning. It is thus shown that J-protein machine is highly conserved.

Keywords J-proteins · Hsp70 · Rice · Transcript expression · Yeast complementation

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Introduction

Proper folding of proteins is central to cell functioning. This is true for the nascent proteins synthesized on ribosomes under optimal cellular conditions as well as for the pre-existing proteins when cells face stress conditions. The adverse environmental conditions are particularly more threatening to plants because of their sessile nature. Network of chaperones monitor and ensure protein quality control and homeostasis in the cells. The chaperones have been classified into various families like Hsp100, Hsp90, Hsp70/Hsp40, Hsp60/Hsp10, and sHsp. In the integrated model of protein surveillance system, Hsp70 chaperone machine collaborates with Hsp100 (Sielaff and Tsai 2010; Miot et al. 2011). Hsp70 is assisted by co-chaperone J-proteins and nucleotide exchange factor (Miot et al. 2011). Together, these proteins constitute a chaperone machine that participates in protein folding, prevention of protein aggregation, translocation of proteins across membranes, targeting proteins towards degradation, and regulation of translation initiation (Kelley 1999; Qiu et al. 2006).

The conserved signature sequence of all J-proteins is the 70 amino acid long J-domain which is mostly present near the N-terminus (Cyr et al. 1992). A highly conserved HPD tripeptide is the characteristic feature of J-domain (Kampinga and Craig 2010). Based on the presence of specific conserved regions, J-proteins are classified into three types. Type I J-proteins are characterized by an N-terminus J-domain followed by a stretch of G/F-rich region, a cysteine rich Zn finger-like motif and may contain a loosely conserved C-terminal region involved in dimerization and substrate binding (Lu and Cyr 1998). Type II J-proteins are similar to type I except that they lack the Zn-finger domain. A universally conserved tripeptide of Asp-Ile/Val-Phe referred as DIF motif in the G/F region is proposed to be critical for J-proteins of *Escherichia coli* (Cajo

et al. 2006). Type III J-proteins are the most diverse group, defined by the presence of the J-domain which may be present anywhere along the length of the protein. The proteins which contain a J-like domain but lack the critical HPD tripeptide are classified as type IV J-proteins (Kampinga and Craig 2010). Some J-proteins contain additional domains like protein disulphide isomerase domain and ubiquitin interacting motifs that impart specific functional roles to these diverse protein family members (Kampinga and Craig 2010; Chapple et al. 2004). J-proteins bind to the ATPase domain of Hsp70 that initiates ATP hydrolytic cycle, which is central to the functioning of Hsp70 (Misselwitz et al. 1998). It has been suggested that the substrate specificity of Hsp70 proteins is largely determined by their J-protein co-chaperones. J-proteins deliver the substrate to Hsp70, an event during which a ternary complex between J-protein, the substrate and Hsp70 is probably formed and subsequently the J-protein component is released (Misselwitz et al. 1998).

Detailed information on J-proteins has accrued from studies on *E. coli* and *Saccharomyces cerevisiae*. *S. cerevisiae* contains 22 J-proteins (Walsh et al. 2004) and there are 6 J-domain proteins (called DnaJ) in *E. coli* (Mayer and Bukau 2005). Ydj1 (YNL064C) is the most abundant cytosolic J-protein and is reportedly required for the normal growth of yeast cells (Caplan et al. 1992). Null mutations of this gene lead to slow growth at 30 °C and inviability at elevated temperatures (Caplan et al. 1992). Ydj1 co-operates with Hsp70 protein Ssa1 in cellular processes like stress response, maintenance of protein quality by protein folding, suppression and rescue of protein aggregates, and translocation of proteins to endoplasmic reticulum (ER) and mitochondria (Caplan et al. 1992; Glover and Lindquist 1998). Miernyk (2001) reported 89 J-proteins in *Arabidopsis*. Recent analysis has reported that *Arabidopsis* genome has 120 J-proteins (Rajan and D'Silva 2009). In plants, J-proteins have been implicated in protection against environmental stresses (Yang et al. 2009; Qi et al. 2011; Zhou et al. 2012; Sung and Guy 2003). J-proteins have also been implicated in developmental programs of the plants. J-proteins TMS1 and EDA3 are implicated in thermotolerance of pollen tubes and development and function of the female gametophyte respectively in *Arabidopsis* (Valencia-Morales et al. 2012; Yang et al. 2009). Specific J-proteins are also found to be essential for *Arabidopsis* growth and their absence has been associated with gametophytic defect and embryo lethality (Yamamoto et al. 2008). J-proteins are shown to act by modulating activity of transcription factors as well. The flowering time in *Arabidopsis* is regulated by AtJ3 protein through its direct binding with a MADS-box transcription factor (Shen et al. 2011). Crystal structures of *Arabidopsis* JAC1 and *Nicotiana* NtCPIP have been determined (Takano et al. 2010; Griessl et al. 2012). CPIP interacts with capsid protein of potyvirus and mutation in CPIP leads to loss in viral infectivity suggesting that J-protein function in plant virus infection and replication (Hofius et al. 2007). Takano et al. (2010) showed that J-domain of JAC1 possesses a positive charge surface that forms a putative interface with Hsp70. Mutation in HPD tripeptide of JAC1 hampered its functional involvement in chloroplast photo-relocation movement (Takano et al. 2010).

We have aimed at developing a model for the J-proteins in higher plant rice, in this study. In earlier attempts, J-protein of rice was shown to play a role in UV-induced DNA damage (Yamamoto et al. 2005) and in interaction with viral movement protein facilitating cell-to-cell movement of virus (Lu et al. 2009). We identified 104 J-protein coding genes in rice genome. Transcript expression analysis revealed that various J-genes are regulated under different stress conditions and the genes expressed constitutively or regulated developmentally. Yeast mutant of Ydj1 protein was employed for functional complementation by J-proteins of rice.

Materials and methods

Identification of J-proteins in rice genome

Hidden Markov model-based DnaJ domain (chaperone J-domain superfamily) entries of *Oryza sativa* (var. *japonica*) genome were retrieved from Superfamily1.75 (http://supfam.org/). As the genome information in Superfamily1.75 was based on Rice Genome Annotation Project (RGAP) 5.0, the retrieved genes were subsequently analyzed in the RGAP release 6.1 (http://rice.plantbiology.msu.edu/). After removing the redundant entries, protein sequences of 125 DnaJ domain containing genes identified in rice genome were downloaded from RGAP. Subsequent analysis of these genes in SMART database to confirm the presence of DnaJ domain led to 123 DnaJ domain containing sequences and two genes (Os01g73020 and Os10g33910) containing PAM16 domain. PAM16 domain is considered as DnaJ-like protein. PAM16 sequences were excluded in tree analysis. Following the strict criteria of HPD motif containing J-domain, 104 genes qualified as J-proteins of rice. Os10g42439 was manually curated based on the available FL-cDNA sequence. Os03g12236 in RGAP coding for 256 amino acid protein showed partial domains of type I DnaJ suggesting incomplete or truncated protein. FGENESH analysis of genomic sequence of Os03g12236 at softberry (www.softberry.com/) revealed presence of one gene having 10 exons coding for 462 amino acid protein. The amino acid sequence deduced from softberry, on analysis in SMART database showed presence of all characteristic domains of type I J-proteins (Electronic supplementary material (ESM) Fig. 1).

Multiple sequence alignment of J-proteins was performed using the Clustal X 2.0 (Larkin et al. 2007) with default parameters. The NJ tree with 1,000 bootstrap was constructed in Clustal X 2.0 and viewed using tree drawing tool FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Subcellular localization of J-proteins was analyzed at WoLFPSORT, Predotar, PSORT, and TargetP database. Consensus localization in two algorithms was considered as the subcellular compartment for the specific entries (ESM Table 1).

Isolation of RNA, RT-PCR analysis, and cloning of FL-cDNA

Total RNA was isolated from stressed and nonstressed (control), 3-week-old rice seedlings [*O. sativa* L; cultivar Pusa basmati 1 (PB1)] using TRI reagent (Sigma, USA) as per the manufacturer's instructions. RNA quantification was carried out spectrophotometrically and the quality of RNA was analyzed by gel electrophoresis. For cDNA synthesis, 5 μg of total RNA of each sample was reverse transcribed using reverse transcriptase (RevertAid H Minus Reverse transcriptase, MBI, Fermentas) and reaction was performed according to manufacturers' protocol. Reverse transcription polymerase chain reaction (RT-PCR) amplification was performed using gene specific primers listed in ESM Table 2.

For yeast mutant complementation and yeast-2-hybrid (Y2H) analysis, J-genes were amplified using FL-cDNA clones procured from Rice Genome Resource Centre, Japan (primers used are listed in ESM Table 2). PCR was carried out using Phusion^{TM}Hi-Fi DNA polymerase in presence of 1 % DMSO in a 50 μl reaction. The inserts digested with specific enzymes (ESM Table 2) were cloned in pAD-GAL4 or pBD vectors. For Y2H, the vectors were transformed in YRG2 cells. For yeast mutant complementation, the genes were cloned in p426 vector (Mumberg et al. 1995) under the control of glyceraldehydes-3-phosphate dehydrogenase (GPD) promoter and transformed in mutant cells. The phenotype of the yeast was scored by incubating the plates spotted with tenfold serial dilutions of yeast cells. Prior to spotting the OD_{600} of yeast cells was normalized to 0.2.

Results

In silico characterization of rice J-proteins

Rice genome contains a large number of J-domain containing proteins. The size of these proteins ranges from 112 (12 kD) to 1,507 (164 kD) amino acids (Table 1). Unlike other Hsp gene families, the molecular mass range of J-proteins is significantly large. This study identified 104 potential J-proteins belonging to 12 type I, 9 type II, and 83 type III (Table 1). Apart from the above 104 J-proteins, rice genome contains 21 sequences which possess J-domain but lack the crucial HPD motif present in the turn between helixII and helixIII (ESM Table 3 and ESM Fig. 2). Out of these 21 proteins, two proteins have PAM16 domain. PAM16 domain proteins are considered as type IV J-proteins or J-like proteins. Rajan and D'Silva (2009) reported four such type IV genes in *Arabidopsis* genome.

Notably, rice J-proteins were spread throughout the rice genome on all the 12 chromosomes. In addition to the signature J-domain, other diverse domains like TPR domain, Fe–S cluster or AT-hook domain provide further specificity to type III J-proteins (Table 1). According to the domain features of the J-proteins, rice types I and II proteins grouped in two separate clades along with *Arabidopsis* J-proteins in the phylogenetic tree drawn on aligned amino acid sequences (Fig. 1). On the other hand, type III J-proteins were scattered on several clades due to the varied arrangement of domains. One of the J-protein, i.e., Os03g62120 was found to have two DnaJ domains. There were 12 pairs of segmental duplicates (Fig. 1) and four genes were tandem duplicates arranged in same orientation on chromosome 3 (ESM Fig. 3) suggesting that duplication event has little contribution in expansion of such a large gene family. The duplicated pairs of genes were present on the same clades and were found to localize to same cell compartments. J-proteins of each type were localized to various cellular compartments (Table 1). In type I, six proteins were localized to cytosol/nuclear, three to chloroplast, one to mitochondria, and consensus localization could not be predicted for two proteins. No J-protein of type I was localized to ER in rice and *Arabidopsis*. In type II, eight proteins were cytosol/nuclear and 1 was ER localized and interestingly no protein was localized to mitochondria or chloroplast, the two endosymbiont organelles. In type III, 55 proteins were cytosol/nuclear, 15 proteins chloroplastic, 7 mitochondrial, and 2 ER localized.

Nomenclature of rice J-genes

J-protein family has not been extensively studied in rice. As random nomenclature of the J-genes could lead to redundant situation, rice J-proteins identified in this study were designated a new nomenclature which is essentially adopted from Ohtsuka and Hata (2000) proposed for mammalian J-proteins. Accordingly, J-domain proteins were classified into OsDjA, OsDjB, and OsDjC where Dj denotes DnaJ domain and A, B, and C represent types I, II, and III, respectively, and prefix "Os" is the source of DnaJ protein, i.e., *O. sativa*. In the original scheme of Ohtsuka and Hata (2000), the type of DnaJ is followed by an Arabic numeral and a lowercase alphabet to denote the chronological order in which the sequence data is deposited in the database and

Table 1 J-proteins of rice

Locus id	Protein name	Length (aa)	DnaJ Domain	*E* value[a]	Additional domains	Localization
Type A						
Os02g43930	OsDjA1	422	8–108	1.96e-34	ZN, DnaJ_C	C/N
Os02g56040	OsDjA2	488	65–176	5.23e-34	ZN, DnaJ_C	CP
Os03g12236	OsDjA3	257	62–166	7.07e-32	ZN, DnaJ_C, TMD	CP
Os03g44620	OsDjA4	418	8–108	6.28e-36	ZN, DnaJ_C	C/N
Os03g57340	OsDjA5	418	8–109	6.28e-37	ZN, DnaJ_C	C/N
Os04g46390	OsDjA6[b]	417	8–105	3.27e-35	ZN, DnaJ_C	C/N
Os05g26902	OsDjA7[c]	448	87–189	3.79e-33	ZN, DnaJ_C	NP
Os05g26926	OsDjA8[c]	448	87–189	3.79e-33	ZN, DnaJ_C	NP
Os06g02620	OsDjA9	443	72–178	9.29e-32	ZN, DnaJ_C	M
Os06g11440	OsDjD10	1293	932–1,001	3.27e-18	ZN, DnaJ_C	C/N
Os12g07060	OsDjA11	420	65–170	2.22e-31	ZN, DnaJ_C	CP
Os12g42440	OsDjA12	468	10–88	3.79e-28	ZN, DnaJ_C	C/N
Type B						
Os01g13760	OsDjB1	350	3–97	1.7e-31	DnaJ_C	C/N
Os01g65480	OsDjB2	328	8–69	5.63e-17	DnaJ_C	C/N
Os02g03600	OsDjB3	390	3–95	5.89e-31	DnaJ_C	C/N
Os02g20394	OsDjB4	350	3–73	1.02e-32	DnaJ_C	C/N
Os05g03630	OsDjB5	323	4–98	1.44e-31	DnaJ_C	C/N
Os05g06440	OsDjB6	348	22–125	7.07e-34	DnaJ_C	ER
Os05g48810	OsDjB7	363	3–73	3.14e-32	DnaJ_C	C/N
Os08g06460	OsDjB8	343	3–113	1.44e-31	DnaJ_C	C/N
Os08g28700	OsDjB9	345	1–105	6.41e-32	DnaJ_C	C/N
Type C						
Os01g01160	OsDjC1	191	50–128	3.93e-23		CP
Os01g06454	OsDjC2	113	43–104	2.36e-16	SP, PAM18	CP
Os01g17030	OsDjC3	151	6–77	2.36e-19	TMD	C/N
Os01g17040	OsDjC4	212	4–75	4.32e-20	TMD	C/N
Os01g25320	OsDjC5	949	782–946	3.79e-37		C/N
Os01g27740	OsDjC6	1009	57–133	1.83e-19		C/N
Os01g32870	OsDjC7	404	17–98	3.01e-27	cc	C/N
Os01g33800	OsDjC8	604	295–389	2.09e-20	cc	C/N
Os01g37560	OsDjC9	381	59–190	2.22e-27	DUF1977	C/N
Os01g42190	OsDjC10	198	9–100	2.09e-25		C/N
Os01g44310	OsDjC11	1473	1,323–1,471	1.05e-35		C/N
Os01g50700	OsDjC12	653	6–102	3.14e-28	dehydrin	C/N
Os01g53020	OsDjC13	343	77–174	6.54e-20	Fe–S cluster	CP
Os01g69930	OsDjC14	745	62–161	1.31e-22	cc	C/N
Os01g74580	OsDjC15	472	347–457	5.23e-31	5 TPR, SP	M
Os02g10180	OsDjC16	477	351–461	3.4e-31	5 TPR, SP	NP
Os02g10220	OsDjC17	283	26–105	9.81e-27		C/N
Os02g30620	OsDjC18	735	56–132	1.06e-23		C/N
Os02g35000	OsDjC19	378	2–102	7.59e-28		C/N
Os02g46640	OsDjC20	122	10–99	9.68e-28		C/N
Os02g50760	OsDjC21	443	28–99	2.62e-26		C/N
Os02g52270	OsDjC22	133	29–117	1.96e-27		C/N
Os02g54130	OsDjC23	272	19–91	1.3e-23		C/N
Os03g04400	OsDjC24	297	7–83	1.7e-15	RRM	C/N
Os03g10180	OsDjC25	607	491–601	2.36e-26		C/N

Table 1 (continued)

Locus id	Protein name	Length (aa)	DnaJ Domain	*E* value[a]	Additional domains	Localization
Os03g15480	OsDjC26	298	23–147	1.05e-20		CP
Os03g18200	OsDjC27	664	25–118	4.58e-28	cc	ER
Os03g18870	OsDjC28	167	21–92	1.22e-24		CP
Os03g20730	OsDjC29	166	54–124	1.02e-22		CP
Os03g28310	OsDjC30	749	62–133	1.83e-23		C/N
Os03g36160	OsDjC31	293	72–133	1.96e-15		C/N
Os03g51830	OsDjC32	239	165–230	3.66e-13	TMD	C/N
Os03g54150	OsDjC33	612	503–603	9.81e-07		C/N
Os03g55360	OsDjC34	506	44–111	1.96e-18	SP	M
Os03g56540	OsDjC35	129	43–103	4.58e-17	PAM18	M
Os03g60790	OsDjC36	269	47–136	2.62e-25		CP
Os03g61550	OsDjC37	261	31–104	1.57e-18		CP
Os03g61730	OsDjC38	726	429–511	1.57e-18	5 TMD	CP
Os03g62120	OsDjC39	478	65–130, 252–317	5.76e-12		C/N
Os03g62130	OsDjC40	277	69–136	2.36e-11		C/N
Os03g62140	OsDjC41	288	71–136	3.4e-12		C/N
Os03g62150	OsDjC42	263	64–126	1.83e-13		C/N
Os04g24180	OsDjC43	682	96–197	1.09e-24	TMD, Sec63, SP	ER
Os04g31940	OsDjC44	730	55–122	4.32e-23		C/N
Os04g57880	OsDjC45	487	60–147	5.63e-19	Fe–S cluster, TMD	CP
Os04g59060	OsDjC46	275	167–269	2.09e-11	SP	M
Os05g01590	OsDjC47	231	162–224	1.22e-11	SP	M
Os05g30130	OsDjC48	368	100–173	5.23e-27	DUF1977	C/N
Os05g31062	OsDjC49	395	176–322	1.98e-22	4TPR	C/N
Os05g45350	OsDjC50	351	11–140	2.75e-21	Fe–S cluster	CP
Os05g46620	OsDjC51	339	2–100	8.64e-31		C/N
Os05g50370	OsDjC52	1424	1,269–1,422	4.32e-36		C/N
Os06g09560	OsDjC53	236	13–107	3.01e-22		C/N
Os06g13060	OsDjC54	436	21–93	1.83e-27		C/N
Os06g34440	OsDjC55	1019	55–132	1.57e-19		C/N
Os06g44160	OsDjC56	143	44–102	1.57e-15		CP
Os07g03270	OsDjC57	238	166–229	5.76e-14	cc	C/N
Os07g09450	OsDjC58	113	43–104	9.42e-17	SP, PAM18	NP
Os07g28800	OsDjC59	270	149–264	6.8e-13		M
Os07g43330	OsDjC60	271	68–146	8.11e-23	SP	CP
Os07g44310	OsDjC61	135	5–70	9.81e-13	TMD	C/N
Os08g35160	OsDjC62	159	7–86	6.15e-23		C/N
Os08g36980	OsDjC63	175	7–83	5.23e-18	ZnF_CSL	C/N
Os08g37270	OsDjC64	397	63–141	5.5e-08	AT_hook	C/N
Os08g41110	OsDjC65	395	2–101	6.28e-30		C/N
Os08g43490	OsDjC66	147	44–117	2.88e-20		CP
Os09g20320	OsDjC67	330	53–125	9.42e-20		C/N
Os09g28590	OsDjC68	197	8–83	1.57e-17	ZnF_CSL	C/N
Os09g28890	OsDjC69	372	63–141	9.42e-08	AT_hook	C/N
Os09g32050	OsDjC70	396	3–101	4.97e-30		C/N
Os10g03610	OsDjC71	254	57–138	3.27e-10		NP
Os10g11012	OsDjC72	373	71–126	0.000389		C/N
Os10g36370	OsDjC73	541	9–84	8.11e-24		C/N
Os10g42439	OsDjC74	1508	1,591–1,631	3.14e-12		C/N

Table 1 (continued)

Locus id	Protein name	Length (aa)	DnaJ Domain	*E* value[a]	Additional domains	Localization
Os11g36530	OsDjC75	291	153–224	7.33e-17		CP
Os11g36960	OsDjC76	1053	52–131	1.83e-20		C/N
Os11g37000	OsDjC77	625	57–129	4.19e-16		C/N
Os11g43950	OsDjC78	889	740–886	1.22e-36		C/N
Os12g15590	OsDjC79	310	38–115	4.19e-25	2 TMD, SP	C/N
Os12g27070	OsDjC80	261	100–173	7.33e-16		M
Os12g31840	OsDjC81	608	7–81	8.64e-22	2 ZnF_C2H2	C/N
Os12g36180	OsDjC82	925	785–923	4.45e-37	cc	C/N
Os12g41820	OsDjC83	545	219–351	4.58e-19	4 TMD	PM

[a] *E* value of DnaJ domain

[b] RNB8 in Lu et al. (2009)

[c] BAB70509, accession number of protein according to Yamamoto et al. (2005) and mapped to chromosome 5. Both DnaJ genes present on chromosome 5 code for exactly same protein, therefore, BAB70509 represents both the genes

DnaJ_C- C-terminal domain, *RRM* RNA recognition motif, *SP* signal peptide, *TMD* transmembrane domain, *cc* coiled coil domain, *TPR* tetratricopeptide region, *PAM* Presequence translocase-associated protein import motor, *DUF* domain of unknown function, *C/N* cytosol/nuclear, *CP* chloroplast, *ER* endoplasmic reticulum, *M* mitochondria, *PM* plasma membrane, *NP* not predicted

the splice variants of the gene, respectively. There are only two reports in literature on J-proteins of rice (Table 1). In this study, the Arabic numbers are depicted on the basis of order in which the J-genes are arranged on the chromosomes (Table 1). Accordingly, the first gene in Table 1 is OsDjA1, where "Os" is source organism *O. sativa*, "Dj" is DnaJ/Hsp40/J-protein, "A" denotes type I, "1" denotes the first protein in type I on chromosome.

Rice J-proteins are induced by heat and other abiotic stresses

Transcript expression profiles of various J-proteins in response to abiotic stresses were analyzed *in silico* from microarray data at Genevestigator (https://www.genevestigator.com/gv/). In addition, expression of selective genes was assessed by semi-quantitative RT-PCR. The results indicated diverse expression patterns for the J-genes (Fig. 2 and ESM Fig. 4). A large number of J-genes were constitutively expressed. The expression profiles as seen by RT-PCR experiments were largely similar to the microarray meta-analysis results (Fig. 2 and ESM Fig. 4). This was especially noticeable in the case of Os01g53020, Os03g18200, Os05g48810, Os06g02620, Os06g09560, and Os11g10990 genes. Several J-genes like Os03g57340, Os06g02620, Os05g48810, Os03g15033, Os01g50700, Os03g18200, Os06g09560, and Os06g44160 were heat stress (HS) inducible. Moreover, a positive correlation between transcript expression levels and the duration of stress was distinctly observed for Os01g25320, Os03g18200 and Os06g09560 in RT-PCR. Most of the type A genes were upregulated under anoxia. In type B, Os05g48810 gene showed a highly induced expression level in almost all the stress conditions except cold stress (CS). Os3g18200 was significantly downregulated in response to CS as evident from RT-PCR as well as microarray data (Fig. 2 and ESM Fig. 4). Os03g56540 and Os06g09560 genes of type C showed an increased expression level under all stress conditions except CS. The genes like Os08g03380, Os09g21250, and Os11g10990 without HPD domain were specifically induced by HS (Fig. 2).

Developmental regulation of J-proteins

Transcripts for Os02g43930, Os03g44620, and Os04g46390 were highly expressed in the dough stage, indicating possible importance of these proteins at the time of seed setting (ESM Fig. 5). Os02g43930 transcript showed relatively high expression in most of the tissues and throughout the development whereas Os04g46390 transcripts showed high expression levels in vegetative tissues. Os03g62120 and Os03g62140 transcripts showed high expression at all the developmental stages and in almost all the tissues (ESM Fig. 4). Os08g06460 and Os02g20394 genes appear to be important specifically in the pollen tissues (ESM Fig. 5). Drawing parallels from a report of a J-protein ortholog that is crucial for thermotolerance in pollen and pollen tube in *Arabidopsis* and thus maintaining male fertility (Yang et al. 2009), similar roles for the pollen-associated J-genes of rice may be proposed.

Rice J-protein functionally complement corresponding yeast mutants

An important approach for functional analysis of stress genes is through analysis of mutants. However, genetic

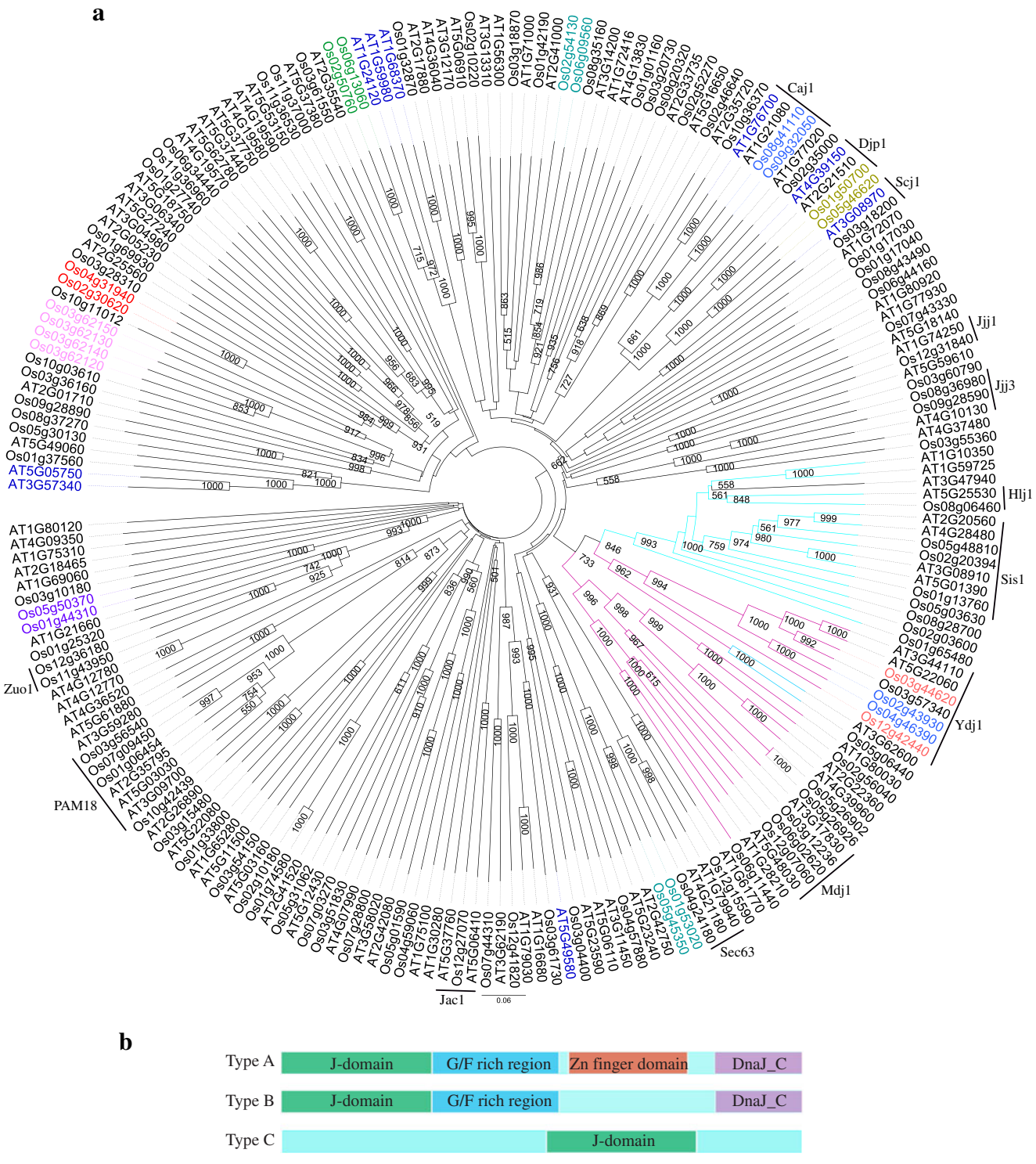


Fig. 1 Phylogenetic tree of J-proteins of rice and *Arabidopsis*. **a** Amino acid sequences of J-proteins of rice and *Arabidopsis* (Rajan and D'Silva 2009) were aligned in Clustal X (2.0). The bootstrap NJ tree was generated in clustal X (2.0) and viewed in FigTree v1.1.1.1. The bootstrap support value (>50 %) is shown at the nodes. Clade in purple depicts type I J-proteins, clade in blue depicts type II J-proteins and black line clades are type III J-proteins. *Arabidopsis* J-proteins in blue font are type II (in Rajan and D'Silva 2009), but cluster in this tree with type III proteins. The segmental duplicated pairs are marked in same color font. **b** Diagrammatic representation of different types of J-domain proteins. In type C, the J-domain can be located anywhere in the protein sequence. In type D, a J-like domain is present

resources of rice are not exhaustively available. In yeast, Ydj1 (YNL064C) is the most abundant J-protein and is reportedly required for the normal growth of yeast cells (Caplan et al. 1992). It is reported that though Ydj1

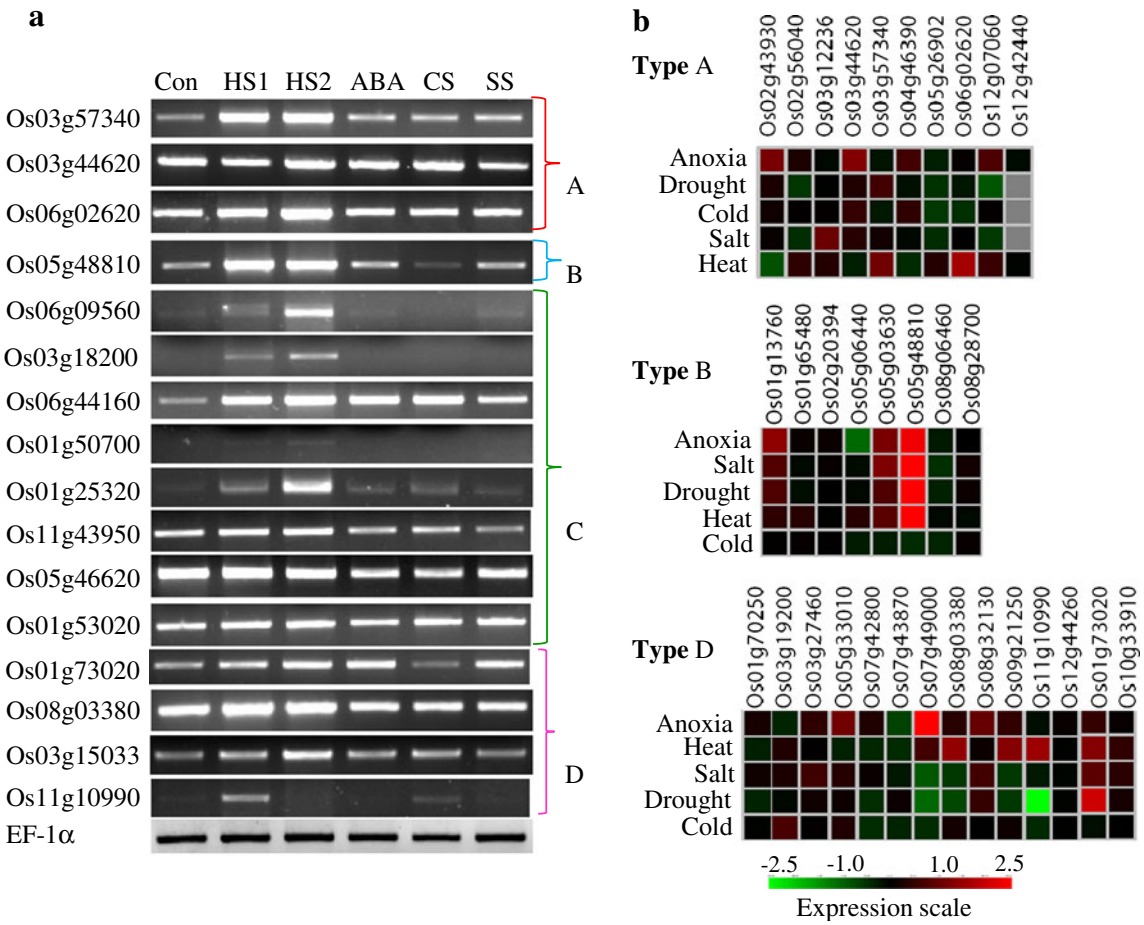
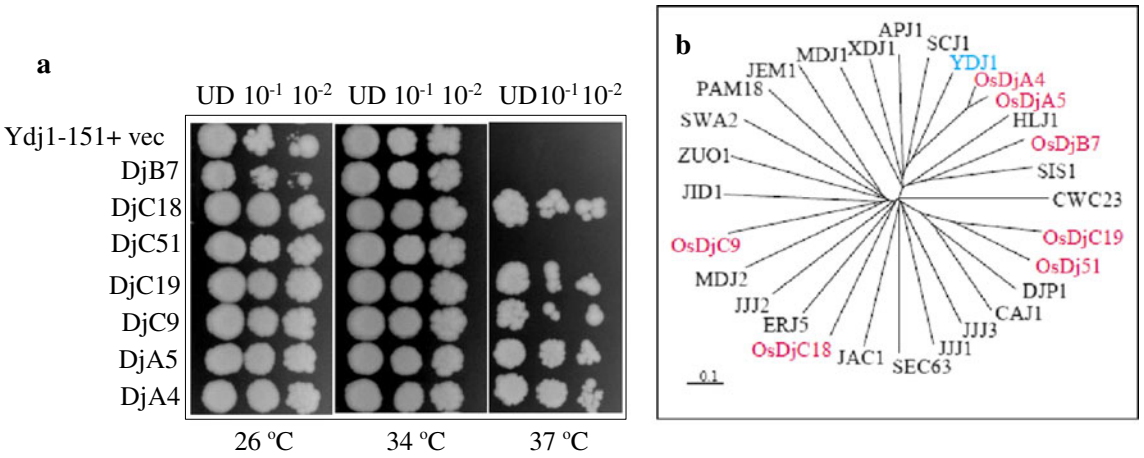


Fig. 2 Expression analysis of selective J-proteins of rice by semiquantitative RT-PCR. **a** For RT-PCR cDNA used was synthesized using 5 μg RNA isolated from rice seedlings stressed as follows: *HS1* heat shock at 42 °C for 10 min, *HS2* heat shock at 42 °C for 60 min, *ABA* 100 μM abcissic acid for 3 h, *CS* cold stress at 6 °C for 6 h, *SS* salt stress in 150 mM NaCl for 6 h. *Con* unstressed control maintained at 26 °C. **b** *In silico* expression analysis of types A, B, and D J-proteins of rice under stress conditions. Expression profiles of type C J-proteins are presented in ESM Fig. 4. Primers used for RT-PCR are listed in ESM Table 2

expression is not heat inducible, mutation in this gene leads to slow growth at 30 °C and inviability at elevated temperatures (Caplan et al. 1992). Orthologous genes of Ydj1 in rice and in *Arabidopsis* (AtJ3; At3g44110) are also strongly expressed. The mutant of this gene in *Arabidopsis* is reported to have late flowering phenotype (Shen et al. 2011). However, no phenotypic aberration was observed in the mutant *Arabidopsis* lines in comparison to wild type after heat stress at various temperatures and stress duration (Fig. 3). Seven rice J-proteins (two type A: DjA4 and DjA5; one type B: DjB7; and four type C: DjC9, DjC18, DjC19, and DjC51) were expressed under the control of GPD promoter in ydj1 cells to analyze if they could complement the growth defect of ydj1 mutant. Notably, the growth defect phenotype of ydj1 at 37 °C was rescued by expression of five out of seven rice J-proteins (Fig. 3). Two genes, DjB7 and DjC51 failed to complement the growth defect at 37 °C. DjB7 expressing yeast cells showed slow growth even at 26 °C. The rice DjA4 and DjA5 were more efficient in rescuing the growth defect at 37 °C as compared to other three genes (Dj18, Dj19, and Dj51). A tree drawn on the aligned amino acid sequences of seven rice J-proteins with yeast J-proteins showed that DjA4 and DjA5 are closest orthologues of yeast Ydj1 (Fig. 3). Hence, selective rice J-proteins can function in yeast and can interact with yeast chaperone machinery.

Physical interaction of rice Hsp70 and J-proteins

Pairwise interactions of J-proteins and Hsp70 were analyzed by yeast-two hybrid assays. Considering that the probability of interaction of proteins localized in the same compartment would be higher, the nucleocytoplasmic localized J-proteins and Hsp70 proteins were analyzed in this study. It is shown that yeast Ydj1 interacts with yeast Hsp70, Ssa1 (Caplan et al. 1992). Hsp70 proteins analyzed in this study included Os03g16880, Os02g60620, Os03g16920, and Os11g47760, and J-proteins included Os01g37560 (DjC9), Os02g35000



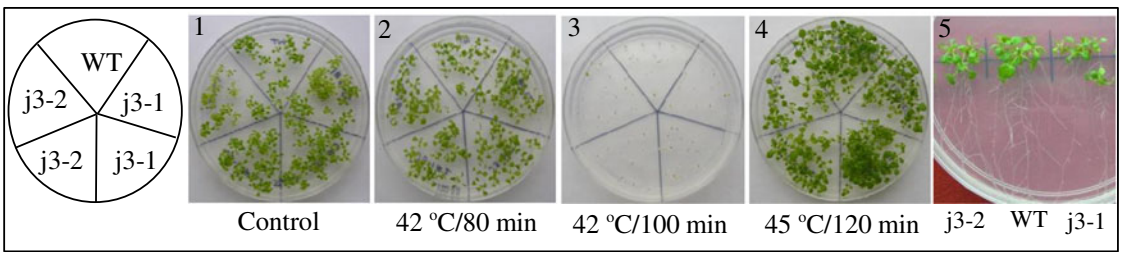


Fig. 3 **a** Complementation of yeast mutant (*ydj1Δ*) by rice J-proteins. *ydj1Δ* (*MATα ade2-1 leu2-3,112 his3-11, 15 trp1-1 ura3-1can1-100 ydj1-2::HIS3 LEU2::ydj1-151*) mutant cells were transformed with specified J-proteins of rice as shown. The cells were grown to log phase, growth was normalized to 0.2 OD_{600}, tenfold serial dilutions were spotted on selection medium and plates were incubated at specified temperatures. UD denotes cells normalized to 0.2 OD_{600}. **b** Phylogeny of yeast J-proteins and rice J-proteins. The tree was generated in clustalX2.0 from aligned amino acid sequences of rice J-proteins used for *ydj1* mutant complementation and yeast J-proteins. The tree was viewed in Treeview1.6.6. **c** Phenotype analysis of j3 mutant of *Arabidopsis*. Seven-day old seedlings of T-DNA insertion mutant lines j3-1 (Salk_132923) and j3-2 (Salk_141625) of *Arabidopsis* J3 protein (At3g44110) were given heat stress at 42 °C for different duration (*1* and *5* unstressed, *2* and *3* stressed; *4* seeds heat stressed). The growth response was scored after 10 days of treatments

(DjC19), Os02g30620 (DjC18), Os05g46620 (DjC51), and Os05g48810 (DjB7). Notably, none of the J-proteins and Hsp70 proteins from the above range yielded positive interaction (results not shown).

Discussion

Previous studies divulged the complexity and functional diversity of J-protein family of yeast and *Arabidopsis* (Walsh et al. 2004; Rajan and D'Silva 2009). Our analysis highlights the diversity of J-proteins at sequence as well as at expression levels in rice. Interestingly, we found that larger genome of rice contains 104 J-proteins as against smaller genome of *Arabidopsis* which has 116 J-proteins. Both in prokaryotic and eukaryotic organisms, J-proteins and Hsp70 chaperones work in conjunction in controlling various cellular processes. Constitutive as well as stress modulated expression of J-proteins in this study suggests that Hsp70/J-protein bichaperone machine of rice may be involved in basal as well as stress-related cellular functions. Some functions of these proteins can be envisioned based on studies in other systems. J-proteins localized to ER may be crucial as proper folding of secretory proteins in ER is essential for the maintenance of protein quality, homeostasis and cell viability (Vembar et al. 2009). Defect in protein translocation in T-DNA insertion lines in ER proteins, AtERDJ2A and AtERDJ5C has a probable effect on pollen germination (Yamamoto et al. 2008; Yang et al. 2009). While cytosolic, ER and mitochondrial Hsp70 and J-proteins functions are conserved in all organisms, functions of chloroplastic J-proteins are unique to plants. In rice, 18 J-proteins are predicted to be localized in chloroplast. Functional association of J-proteins and Hsp70 was revealed in the biogenesis of thylakoid membranes in

Chlamydomonas (CDJ2/Hsp70B; Liu et al. 2005) and movement of chloroplast in *Arabidopsis* that has implication in photorelocation of chloroplast (Suetsugu et al. 2010). JAC1, the J-protein involved in movement of chloroplast, was functionally impaired if its HPD tripeptide was mutated to AAA (Suetsugu et al. 2010). It will be worthwhile to assess the role of 21 J-proteins in rice that do not contain HPD motif. Further, Fe–S cluster containing proteins in *Chlamydomonas* function as redox switches (Dorn et al. 2010). Three Fe–S cluster containing J-proteins are present in rice (OsDjC13, OsDjC45, and OsDjC50) and like in *Chlamydomonas*, expression of these genes was downregulated under heat stress. Based on similarity in the expression pattern and localization, it may be argued that Fe–S cluster containing J-proteins in rice are also involved in redox sensing. The expression patterns of J-genes in specific cellular compartments differed drastically, suggesting their functional diversity. Functional diversity of J-proteins is further noted from the expression profiles of 4 J-proteins of type A containing –CAQQ sequence at the C-terminus. All these four proteins (DjA1, DjA4, DjA5, and DjA6) showed distinct developmental and stress regulation. However, expression of only DjA5 was elevated at high temperature. The spatial expression of all 4 above proteins was differential during developmental stages.

The functionality of J-protein machinery of rice was analyzed in this study by expressing 7 rice J-domain proteins in ydj1 mutant. Both *E. coli* and yeast mutants have been used for functional analysis of J-proteins from evolutionary diverse species (Nicoll et al. 2007; Vembar et al. 2009). This study shows that the functions of yeast cytosolic Ydj1 could be performed by expression of diverse J-proteins of rice. Ydj1 protein of yeast belongs to Type A class that contains an N-terminal J-domain, a Zn-finger domain and a C-terminal domain. In addition, Ydj1 contains a conserved -CAAX box motif at C-terminal end, which is reportedly a site for farnesylation. The latter reaction promotes association of these proteins to the cytosolic side of the ER membrane (Caplan et al. 1992). Caplan et al. (1992) have suggested that this post-translational modification is crucial for the function of yeast Ydj1 protein at elevated temperature. Rice DjA4 and DjA5, structurally similar to Ydj1 performed more efficiently than other J-proteins tested in this study in ameliorating the growth defect of ydj1 mutant at high temperature. Two rice J-proteins, DjB7 and DjC51, however failed to rescue the growth defect of yeast Ydj1 mutant. This failure in functional complementation could be due to specificity of J-proteins for their partner client proteins (Vembar et al. 2009). The other plausible reason could be difference in cellular localization of the above proteins (Sahi and Craig 2007). The rescuing of growth defect by selective J-proteins of rice indicates their capability to stimulate the ATPase activity of yeast Hsp70 as reported for other systems (Nicoll et al. 2007; Vembar et al. 2009). Moreover, it shows that sequences other than J-domains can also dictate the functional specificity of J-proteins (Sahi and Craig 2007; Hennessy et al. 2005). In addition, domain swapping studies revealed that J-domains of all J-proteins cannot functionally substitute for each other (Hennessy et al. 2000; Kluck et al. 2002; Nicoll et al. 2007). Further experiments are required to narrow down on the sequences other than J-domains for function of J-protein.

Like in other organisms, rice J-domain proteins are one of the largest and most diverse family of chaperones. It is thus suggested that multiple J-proteins can pair with single Hsp70. The pairwise interaction analysis of selected cytosolic J-proteins and Hsp70 by yeast-2 hybrid analysis did not show positive interaction in this study. It is possible that Hsp70 and J-proteins do not interact directly. There are different views proposed on the interaction of Hsp70 with J-proteins. DnaK and DnaJ were demonstrated to bind to different sites of the same substrate with substrate binding domain forming a ternary complex (Han and Christen 2003). Terada and Oike (2010) confirmed independent binding of Hsp70 and J-proteins to unfolded proteins to distinct segments of the peptide. On the contrary, direct physical interaction of Hsp70 to J-protein (both mitochondrial) was reported recently (Zhou et al. 2012). Furthermore, we predict that ~80 J-proteins (this study) and 11 Hsp70 genes (unpublished data) are localized to cytosol/nucleus compartment. Considering such high numbers of cytosolic J-proteins and Hsp70 members, the appropriate pairing may have been missed with limited J-proteins used in pairwise interactions carried out in this study. We aim to study Hsp70 and J-protein interaction by screening cDNA library of rice with different rice Hsp70 as baits in future course.

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